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SURFACTANT ENHANCED MICROBIAL DEGRADATION OF JP-8 CONTAMINATED SOIL

John D. Thomas, Captain, USAF

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CONTAMINATED SOIL

THESIS

John D. Thomas, B.S.

Captain, USAF

December 1996

Presented To The Faculty of The School Of Engineering

of the Air Force Institute of Technology

Air University

In Partial Fulfillment of the

Requirements for the Degree of

Master of Science in Engineering and Environmental Management

Prof. Daniel E. Reynolds

Dr. Mark N. Goltz, PhD

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John Thomas

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Abstract

This research effort used an automated respirometer to evaluate the enhancement of JP-8 fuel biodegradation, from surfactant addition. Two nonionic surfactants, Novell II 1412-7 and Alfonic 810-4.5 were chosen for addition to contaminated JP-8, microcosms at three levels of treatment. The three levels of treatment included sub-CMC, CMC, and supra-CMC concentrations for each surfactant. The Novell II, nonionic surfactant, exhibited inhibitory characteristics, and was eliminated from the study. The focus of the study was to determine if JP-8 was actually degraded, and if enhancement of JP-8 biodegradation would be achieved from surfactant addition. The respirometer was found to be repeatable within experimental runs. However, reproducibility between experimental runs was not as easy to conclude. The reason for this was that measurement error was introduced from adding surfactants from separate stock solutions. JP-8 biodegradation was proven at all sampling intervals using a statistical hypothesis test about the difference of the means. Enhancement, inhibition, or no effect of surfactant addition was concluded using a statistical hypothesis based on the difference of the mean oxygen uptake, at each sampling interval, for the combined treatments of JP-8 and surfactant, and the individual treatments of JP-8 and surfactant. One level of Alfonic surfactant addition, CMC, resulted in enhancement being concluded. The supra-CMC level of Alfonic surfactant addition resulted in a conclusion of inhibition over the first 60 sampling intervals, while each of the remaining intervals resulted in a conclusion of no effect. The sub-CMC levels of Alfonic surfactant addition were concluded to have no effect on the biodegradation of JP-8, at all sampling intervals.

SURFACTANT ENHANCED MICROBIAL DEGRADATION OF JP-8 CONTAMINATED SOIL

1. INTRODUCTION

1.1 OVERVIEW

Anthropogenic sources have contaminated subsurface soils and ground water aquifers. Fuels from underground storage tanks, solvents from industrial processes, and petroleum products from refineries are examples of contaminants which threaten groundwater quality. The cleanup of contaminated sites has been slow and expensive. *In situ* bioremediation may be a good alternative for cleaning up sites, however the time frame for cleanup by this method can be lengthy as a result of hydrophobic organic compounds becoming sorbed to porous media where microorganisms cannot access them for biodegradation. One alternative to increase the bioavailability, and hence, the biodegradation rate, involves the use of surfactants, to increase the rate and extent of desorption and solubilization.

1.2 PROBLEM

When Hydrophobic Organic Compounds (HOCs) are present in the subsurface they have a tendency to become sorbed to porous media where microorganisms cannot access them for transformation into simpler forms. Therefore, the focus of this study is to determine if hydrophobic compounds can be solubilized through the use of surfactants in order to increase their bioavailability and therefore, their biodegradation.

1.3 PURPOSE

The purpose of this experimental study is to determine the enhancement/inhibiting effects of surfactants on microbial degradation of JP-8 fuel under simulated spill conditions. Microbial metabolism will be measured with a Micro Oxymax respirometer. Baker (1995) and Totten (1995) both demonstrated, with respirometry, that when different types of soil are artificially contaminated with JP-8 petroleum fuel, the indigenous organisms are able to metabolize and degrade the petroleum fuel. This study will parallel the work of Baker (1995) and Totten (1995), but will focus on only one soil type which has a small fractional organic content. Surfactants will be added at different concentrations to determine the effect of sub critical micelle, critical micelle, and supra critical micelle concentrations on the amount of oxygen uptake by the soil microbial populations. The primary goals of this study will be to determine:

- JP-8 concentrations which support microbial metabolism, as measured by oxygen uptake of soil microcosms.
- Surfactant concentrations which inhibit and/or enhance microbial metabolism of JP-8 petroleum fuel.
- Metabolic enhancement/inhibition as a function of surfactant type and concentration.

1.4 Terms Used in this Study

Biodegradation -	The breakdown of organic compounds to simpler forms by microbial metabolism.
CMC -	Critical micelle concentration (CMC) refers to the concentration of surfactant in a solvent where surfactant monomers begin to associate to form micelles.
Hydrophobic - Organic Compounds (HOC)	Nonpolar organic compounds with low solubilities in aqueous solution.

JP-8 - A kerosene based hydrocarbon jet fuel used primarily by the United States Air Force.

Metabolism - Chemical changes within living cells by which energy is provided for microbial growth and the necessary maintenance of cell life.

Micelle - Association of surfactant monomers typically in the shape of a sphere, with hydrophobic groups pointed inward and their hydrophilic groups pointing towards the aqueous solvent (refer to Figure 2.3).

Microorganisms -Organisms that exist naturally in the environment such as : Bacteria, Fungi, or Viruses.

Mineralization - Ultimate transformation of organic contaminants to carbon dioxide and water.

Respirometry - The measurement of the oxygen uptake and carbon dioxide evolution associated with biological or chemical systems.

Sorption - Chemical process whereby dissolved molecules or substances become associated through molecular interactions with solid phases. The molecular interactions can include van der Waals and dipole-dipole interactions, hydrogen bonding, or ionic interactions between charged species. Since hydrophobic compounds favor a non-aqueous phase, they tend to be absorbed by organic matter.

Surfactant - Amphiphilic compound (hydrophobic and hydrophilic ends) that is absorbed at interfaces and reduces the interfacial energy required for the interface to expand. When surfactants are added to water, they tend to associate at the air/water interface.

Transformation - A chemical reaction that occurs chemically or biologically by means of oxidation or reduction processes.

2. LITERATURE REVIEW

2.1 Introduction

In the past decade the USAF has spent billions of dollars cleaning up contaminated ground water and soils. Even with this large expenditure, cleanup efforts are not meeting environmental health risk goals in a timely manner. Faced with large costs and cleanup times, there has recently been more of a focus towards using *in situ* remediation technologies. Bioremediation has emerged as a viable remedial alternative; however the time frame required for transformation of highly sorbed organic contaminants is long. The primary reason for the long time frames is that, due to sorption onto aquifer material, the contaminants are not bioavailable for transformation to simpler, less harmful inorganic compounds. To increase bioavailability requires increasing the apparent aqueous solubility of the contaminant, or increasing its desorption mass transfer rate. One possible way to solubilize and desorb organic contaminants is to add surfactants to the contaminated soil/water system to allow for microbial metabolism of the compounds.

2.2 Biodegradation of Petroleum Hydrocarbons

Biodegradation is a transformation process whereby microorganisms metabolize carbon compounds as food and energy sources. Many organic compounds have been shown to be metabolized by indigenous microorganisms (Song *et al.*, 1990; Bossert and Bartha, 1984; Dibble and Bartha, 1979; Ward *et al.*, 1989; Riser-Roberts, 1992). Table 2.1 lists

organic contaminant classes that have been shown to be biodegraded. Microorganisms exist naturally in water/ soil systems and are diverse in population structure.

Table 2.1 Classes of Organic Contaminants Known to Be Susceptible to Microbial Metabolism

n-Aromatics	Xylenes	Polycyclic Aromatics
n-Alkanes	Alkylbenzenes	PAHs
n-Alkenes	Nitro-substituted Aromatics	PNAs
Phenols	Halogenated Aliphatics	Cycloalkanes
Halogenated Aromatics	PCBs	Branched Alkanes

In the deeper areas of the unsaturated zone there are 10^6 to 10^7 organisms per gram of dry soil (Mitchell, 1992:291). Organic compounds are oxidized by the microorganisms which remove a hydrogen electron to simplify the compound structure. Oxidation occurs aerobically in the presence of oxygen, or anaerobically if there is a sufficient quantity of other electron acceptors present. Typical electron acceptors in anaerobic systems are nitrate (NO_3^-), sulfate (SO_3^{2}), and carbon dioxide (CO_2). The focus of this work is on aerobic processes.

Degradation or metabolism is a chemical/energy transformation which conforms to the first law of thermodynamics. The first law of thermodynamics requires that the energy of the products and reactants be equal. Energy for metabolism is achieved through a combination of exothermic and endothermic reactions. An example of an exothermic reaction is the oxidation of glucose where 686 kilocalories (kcal) of energy are produced along with carbon dioxide and water:

$$C_6H_{12}O_6 + 8O_2 \rightarrow 6CO_2 + 12H_2O + 686kcal$$

The second law of thermodynamics requires that a portion of energy produced, entropy, be unavailable to do work and released in the form of heat. Therefore only a portion of the energy produced will be available for microbial metabolism and sustenance. Microorganisms also utilize endothermic reactions to store energy in intermediate compounds such as adenosine triphosphate (ATP). By storing this energy, the living organisms are able to synthesize compounds that require more energy than what is available through exothermic reactions alone. Microorganisms require that hundreds of these reactions occur simultaneously to support basic cell requirements. Since the rate of these reactions is slow at the environmental living conditions that support cell life, catalysts must be provided to support microbial metabolism.

The catalysts for the transformations to occur rapidly are enzymes. Enzymes, protein molecules produced by living microorganisms are highly specific, but may catalyze reactions of a similar nature. Enzymes are composed of amino acids which are connected in specific and precise ways (Brock and Madigan, 1994:5). The sequence of amino acids determines the structure and catalytic specificity. Microorganisms produce a diverse number of enzymes, which are crucial to cell life. Constitutive enzymes are continuously synthesized to support basic cell growth, while others are induced as required for substrate consumption. When a food source (organic compound) disappears and another source appears, the cells have the capability of adapting to the new source through enzyme induction. Induction of enzymes is controlled by initiating mRNA and activating an operon at the end of a DNA chain (Brock and Madigan,1994:170-172 and Chapelle,

1993:78-80). An operon is a portion of a cells DNA that codes for specific proteins. The specific proteins produced are what initiates the production of the necessary enzyme for substrate transformation. Thus, when foreign substrates are introduced, a microorganism can adapt its metabolism by inducing the necessary enzymes to transform the substrate.

It has been shown that petroleum hydrocarbons are readily biodegradable (Peters *et al.*, 1992; Song, 1990; Bossert and Bartha, 1984; Dibble and Bartha, 1979; Ward *et al.*, 1989; Riser-Roberts, 1992). Petroleum hydrocarbons are complex mixtures containing n-alkanes (paraffins), branched alkanes, cycloalkanes, and aromatic hydrocarbons (See Figure 2.1). Most of the compounds which make up petroleum have a low solubility in water, with exception of the light aromatics-benzene, toluene, ethylbenzene, and xylene (BTEX). BTEX accounts for only 2% to 3% of fuels as a whole. Most of the petroleum hydrocarbons are nine carbons or more.

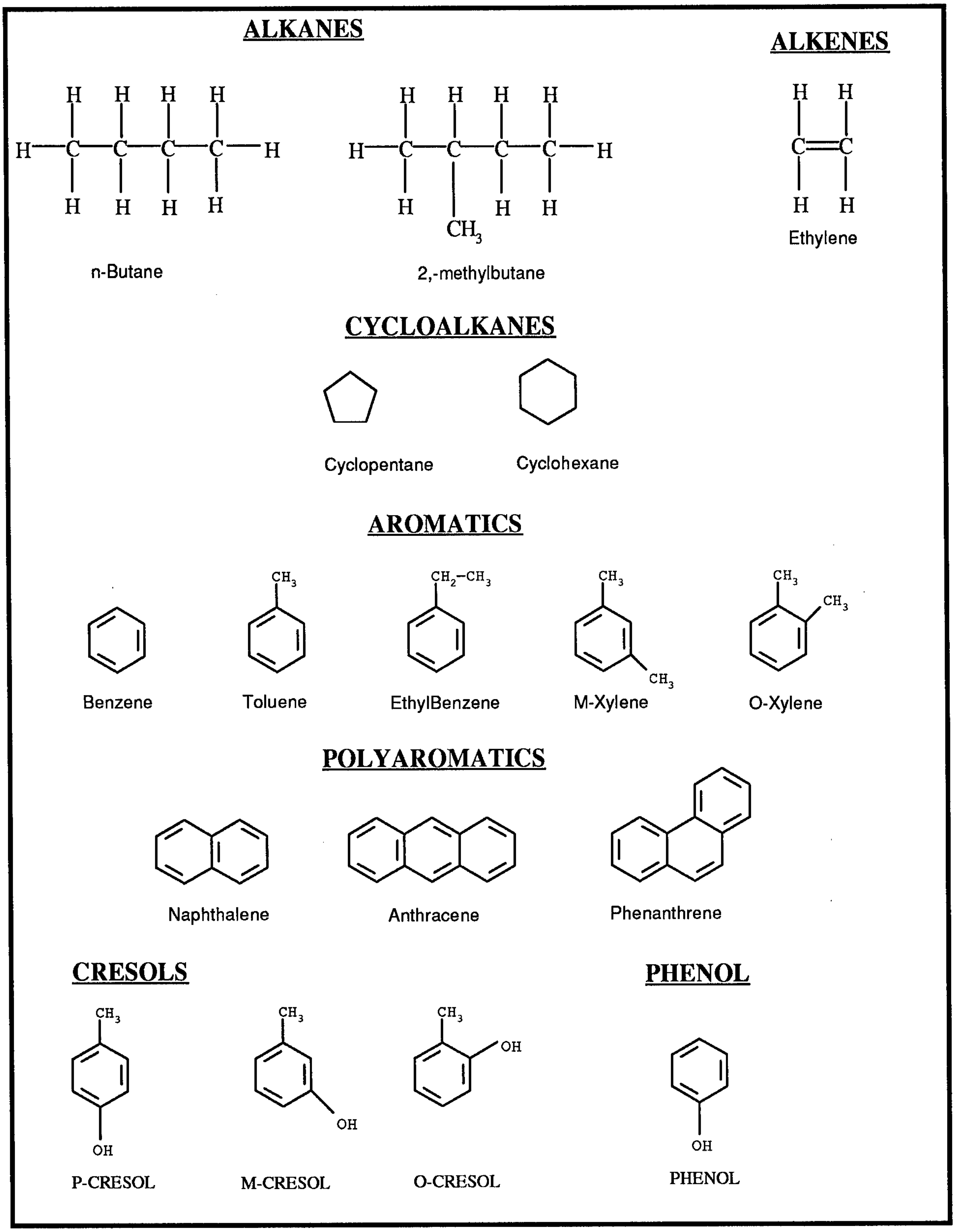


Figure 2.1 Some of the organic compounds that can be found in petroleum fuels (Chapelle, 1993:324).

The bioavailability of the components of fuels will depend on their chemical properties (Table 2.2) and the characteristics of the site (Table 2.3). The more soluble organic contaminants, such as benzene and toluene, are transformed more rapidly than the less soluble organic contaminants, such as naphthalene and phenanthrene, assuming inhibition by toxicity is not a problem.

Table 2.2 Chemical Properties of the Compound

pH	Solubility	Bioavailibility
K_{ow}	Volatility	Chemical Structure
Length of Hydrophobic Chain	Sorption/Desorption Properties	Halogenation of Contaminant
Mass Transfer Rate	Toxicity	Quantity of Contaminant

Table 2.3 Characteristics of the Site

Soil Permeability	Soil pH	Microbial Diversity
Soil Conductivity	Soil Temperature	Bioavailable Substrate
Porous Media	Moisture Content	Redox Potential
Homogeneity of Subsurface	Oxygen Content and Diffusion	Sufficient Electron Acceptors
Soil Organic Matter Content (f_{oc})	Subsurface Nutrient Availability	

Also, in general, straight chain compounds are more easily transformed than branched compounds. The degradation of fuel components is typically started by a monooxygenase enzyme which transfers hydrogen electrons from the compound. After the initial oxidation step, the constituents are further oxidized by other enzymes until ultimate mineralization of the organic compound to carbon dioxide and water. The steps required to reach

mineralization are numerous, and require a significant amount of time. The initial transformations required to degrade benzene are illustrated in Figure 2.2.

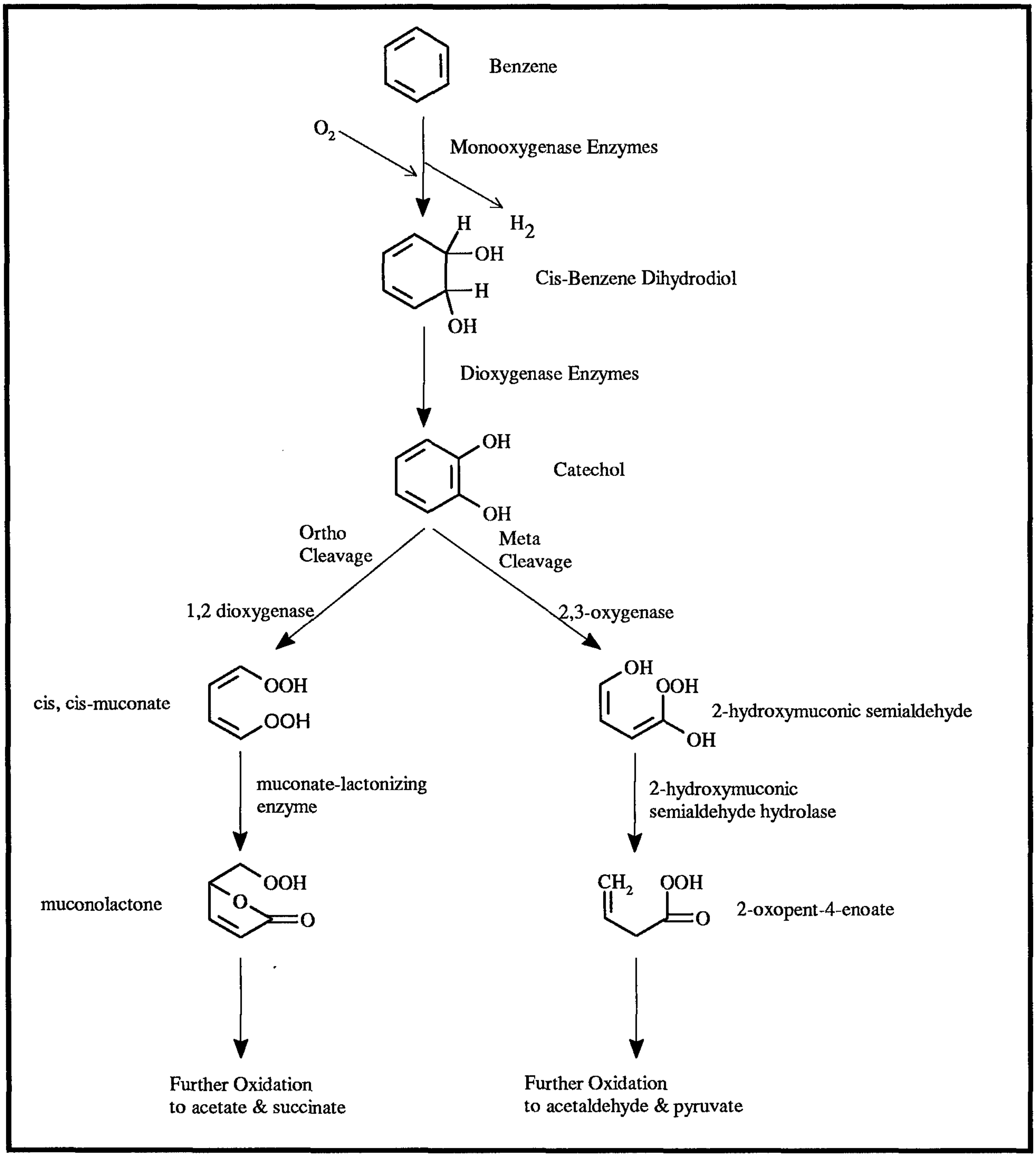


Figure 2.2 Aerobic Degradation of Benzene (Schwarzenbach *et al.*, 1993:496 and Gottschalk, 1986:160-161).

2.3 SURFACTANTS

The use of surfactants to enhance the bioremediation of contaminated environments has been of considerable interest in recent years. Functioning as emulsifiers or solubilizers, surfactants have the ability to increase aqueous concentrations of poorly soluble compounds and interfacial areas between non-aqueous phase liquids and water, thus potentially improving the accessibility of insoluble compounds to microorganisms. Many bacteria under the right conditions can produce biosurfactants and bioemulsifiers to help them metabolize specific compounds. Commercial surfactants can also serve to enhance the dissolution of immiscible hydrocarbons in order to make them bioavailable. Unfortunately, on occasion surfactants have proven to be inhibitory to microbial metabolism (Aronstein *et al.*, 1991; Laha and Luthy, 1991). As a result, surfactants must be carefully selected for remedial alternatives. With proper selection, surfactants can be used in pump and treat systems to increase the concentration of hydrophobic organic compounds in the aqueous phase, thus increasing their mobility and bioavailibility.

Surfactant is a contraction of the term surface-active agent. It refers to an organic compound that has the ability to change the interfacial free energy at interfaces or surfaces. The interface is the boundary between any two immiscible phases. A surface is where at least two separate phases interface, such as a solid and gas (air) or liquid and gas. The interfacial energy is the minimum amount of work required to maintain an interface of a system. When surface tension of a liquid is measured, it represents a measure of the interfacial energy per unit area of the boundary between the liquid and air above it. A

surfactant is therefore a substance that is absorbed at the interfaces, and reduces the interfacial energy required for the interface to expand.

Surfactants have an amphipathic structure, with a hydrophobic and a hydrophilic group (Rosen, 1989:1). When the surfactant is dissolved in a solvent it tends to increase the free energy of the system, decreasing the overall surface tension of the solvent (water). The free energy of the system is increased due to the distortion of the water structure by the surfactant hydrophobic group. As a result, less work is needed to bring the surfactant molecules to the interfaces present in the system. Surfactants can be classified into four basic types depending on the nature of the hydrophilic group of the molecule: anionic, cationic, zwitterionic, and nonionic. Anionic surfactants have a negative charge on the hydrophilic group, whereas a cationic surfactant has a positive charge. Nonionic surfactants have no apparent ionic charge, while zwitterionic surfactants can possess both positive and negative charges on the hydrophilic group. If a surface that is negatively charged is to be made hydrophobic, then a cationic surfactant would be the best type to utilize. Burris and Antworth (1992) showed that significant retardation of perchloroethylene (PCE) and napthalene was possible when aquifer material was treated with a cationic surfactant. Aquifer material, from Columbus AFB, MS, was treated with a cationic surfactant, HDTMA, which resulted in the sorption coefficients of PCE and napthalene being increased over two orders of magnitude. This type of surfactant will absorb onto the negative aquifer material surface with its positive hydrophilic group, thus leaving its hydrophobic group facing out from the surface, making the surface hydrophobic and thereby enhancing sorption and retardation of any HOC present in the

water. If the surface is positively charged, then anionic surfactants will make the surface water repellent. Zwitterionic surfactants tend not to affect the surface charge since they possess both negative and positive charges. Nonionic surfactants absorb onto surfaces with either the hydrophobic or hydrophilic group, depending upon the dominant bonding present at the surface. If hydrogen bonding is present then the hydrophilic group will attach to the surface making the surface hydrophobic, otherwise the hydrophobic group will attach to the surface making the surface hydrophilic. Nonionic surfactants have been the main type used for increasing the amount of HOC that can be solubilized (Edwards *et al.*, 1991; Peters *et al.*, 1992; Edwards *et al.*, 1992; Deitsch and Smith, 1995). Depending on which type of surfactant is absorbed at the surface, the surface charge could be altered and the hydrophobic/ hydrophilic nature of the surface could be changed.

The oil industry uses surfactants to enhance the recovery of crude oil from wells. The initial stage of oil recovery is to pressurize the field. As a result of the pressure, oil in the larger pores will be forced to move with the induced pressure gradient towards the well. About 20% of the oil present is recovered in this initial stage. To increase the pressure gradient further, water is added to the reservoir, forcing the oil to the well. This secondary stage removes up to an additional 20% of the oil present. The remaining oil is trapped in the smaller pores of the rock or soil, by the interfacial free energy or capillary forces. This residual oil that remains requires greater forces to extract. One way to quantify the force required to extract this residual oil is the capillary number C_n (Oh and Slattery,1979). The equation for the capillary number is as follows:

$$C_n = \mu\eta\frac{q}{\gamma} \tag{2-1}$$

where:

μ = fluid (oil) viscosity

η = porosity of the soil or rock

q = cross-sectional flow rate of the water

γ = interfacial tension between the oil and water

As the capillary number increases, the recovery of oil is enhanced (Oh and Slattery, 1979). Therefore, to increase the oil recovery the capillary number must be increased by increasing the flow rate of the water, or decreasing the tension of the oil/water interface.

This leads to the third stage of oil recovery which uses a surfactant to affect the capillary number by decreasing the interfacial tension between the water and oil. The interfacial tension can be decreased from 30 dyne/cm for oil/water under normal conditions to as low as 10^{-4} dynes/cm with the correct surfactant (Peters *et al.*, 1992:115). The wettability of the soil or rock also plays an essential role in removing the oil from the trapped pores. With a water wettable surface, the oil becomes more mobile allowing for more to be recovered. Wettability is another important property of surfactants which enhances the oil recovery process. The wettability of solids/soil can be increased as a result of surfactants creating a hydrophilic soil surface, allowing for water to wet the soil. Surfactants have

been widely used in tertiary oil recovery systems, and the very same properties that enhance oil recovery may help to remediate contaminated soil and groundwater.

2.4 SURFACTANT ENHANCED SOLUBILIZATION/DESORPTION

It has been demonstrated that addition of surfactants can enhance the amount of mineralization (Aronstein *et al.*, 1991), however the exact mechanisms whereby mineralization is increased are not clear. The presence of nonionic surfactants can affect the distribution of hydrophobic organic compounds in soil/ aqueous systems (Edwards and others, 1992:147). When nonionic surfactant is present at a concentration below the CMC, the organic compounds can exist in four states (refer to Figure 2.3): absorbed to the soil, dissolved in water, attached to surfactant molecules in the aqueous phase, or attached to surfactant molecules absorbed to the soil. Whereas, when the concentration of surfactants is large enough to allow for the formation of micelles, the organic compounds may also partition into the hydrophobic interiors of the micelles. Refer to Section 2.4.1 for information on micelle formation.

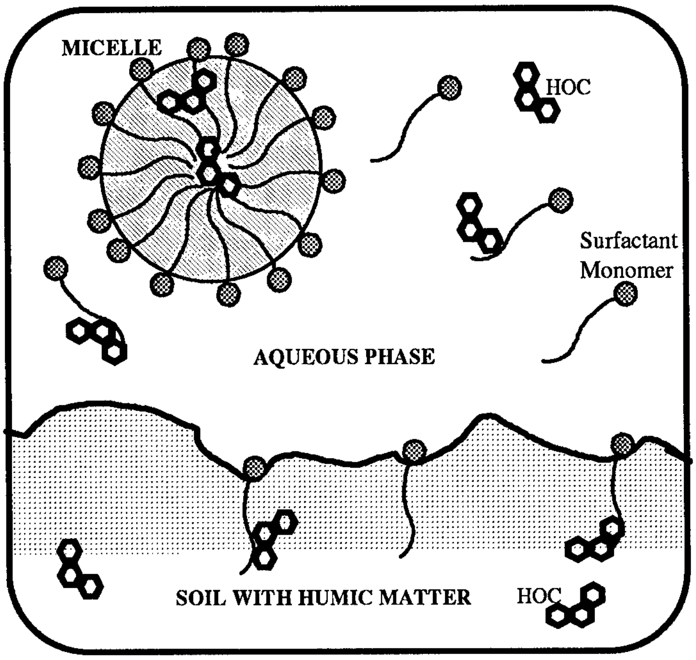


Figure 2.3 An HOC contaminated soil/aqueous system treated with a nonionic surfactant. Schematic represents the different partitioning effects that can result, depending on the properties of the surfactant and the contaminant (adapted from Edwards *et al.*, 1992).

The distribution coefficient for a HOC can be affected by the amount of surfactant sorbed onto the soil surface. Thus as more surfactant is absorbed to the soil, the more surfactant will have to be added in order to obtain a given aqueous surfactant concentration. There are three possible sorption processes for surfactants which include: partitioning into the bulk humus of the soil, formation of a film at the interface, or absorption onto mineral components of the humic material (Edwards *et al.*, 1992). Sorption of surfactants by soil organic matter modifies the fractional organic content of soil, and affects the amount of HOC that can be desorbed and solubilized in the aqueous phase (Edwards *et al.*, 1992:155).

2.4.1 Micellization

The bulk properties of surfactants are unusual, as indicated from the apparent rapid property changes as the concentration of surfactant increases past a certain point (refer to Figure 2.4). Surfactant monomers mixed with water tend to associate, and form micelles, as the concentration of surfactant is increased past a certain point. When a physical property measurement, such as surface tension, conductivity, interfacial tension, or turbidity is plotted as a function of surfactant concentration, discontinuities and rapid increases or decreases in the property measured are seen. The sudden changes in the measured properties indicate a change in the nature of the solution (Myers, 1990:307).

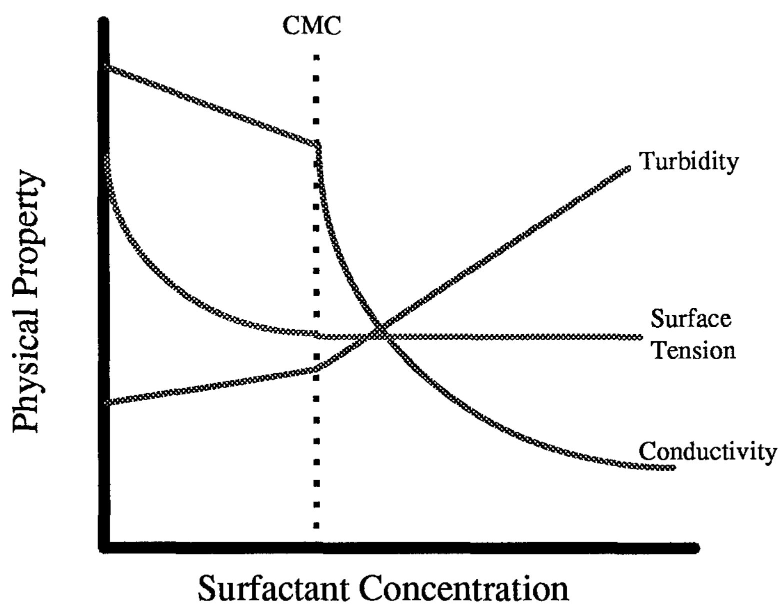


Figure 2.4 Some of the physical property discontinuities, and rapid increases or decreases in the property measured which are seen at the Critical Micelle Concentration (CMC). At the CMC the following changes in physical properties are seen: an abrupt downward change in the solution conductivity, a sharp increase in solution turbidity, and a discontinuity in surface tension (Myers, 1990:306).

The concentration of the surfactant where the discontinuities in physical properties are encountered is known as the critical micelle concentration (CMC). Rosen (1989:108) states that:

> "Micelle formation, or micellization, is an important phenomena not only because a number of important interfacial phenomena, such as detergency and solubilization, depend on the existence of micelles in solution, but because it affects other interfacial phenomena, such as surface or interfacial tension reduction, that do not directly involve micelles."

In a simple surfactant system, surfactant monomers tend to associate with their hydrophobic ends pointing inward; resulting in a hydrophobic interior core and a hydrophilic exterior (See Figure 2.5). The shape that the micelles form depends on the structural makeup of the surfactant, additives present in the liquid phase, change in temperature of the aqueous phase, and change in concentration of surfactant in the aqueous phase. The size of the interior of the hydrophobic core is primarily determined by the length of the hydrophobic head groups on the surfactant monomers. As shown in Figure 2.5 below, the common shape of a micelle is a sphere. An aqueous phase surrounds the micelle, interacting with the hydrophobic head groups and penetrating slightly into the interior of the micelle past the first few methylene groups of the hydrophobic chain. Micelles allow for HOCs to partition into their interior, and can in this respect be considered a separate phase altogether.

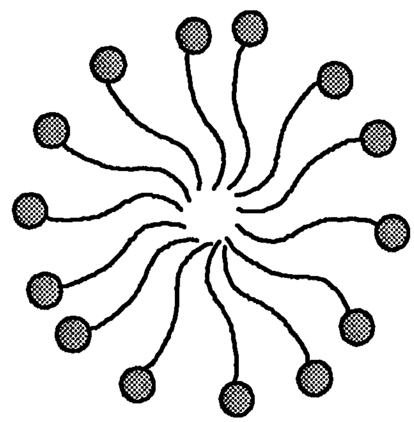


Figure 2.5 Schematic representation of a micelle, spherical in shape, with a hydrophobic interior and a hydrophilic exterior (Rosen, 1989:174).

Solubilization of HOCs by micelles is the primary reason for their use in enhancing removal of contaminants that have a relatively low solubility in water. Micelles can be considered a separate phase as described by the phase separation model (Myers, 1990:307-310).

2.4.2 Solubilization

Solubilization is directly related to micelle formation, and is the primary means of increasing bioavailability. It can be defined as a stable solution of a substance which is normally insoluble in a solvent. Edward's *et al.* (1991 and 1992) have done numerous studies on nonionic surfactants, and found increased apparent solubilities of HOCs when surfactants were added to contaminated soil systems. Solubilization of HOCs is initiated when surfactants are present above their CMC in the aqueous phase. It has been shown that only small amounts of HOC are solubilized below the CMC, but as the concentration of surfactant is increased past the CMC, solubility has an apparent linear increase (Rosen, 1989:171). Solubilization occurs at a number of sites within a micelle (Figure 2.6): (1) the inner core, (2) the palisade layer of the micelle, or the outer core of the micelle between

the hydrophobic groups and the first few methylene groups, (3) the surface of the micelle, and (4) in non-ionic type surfactants, between the hydrophilic groups (Rosen, 1989:172).

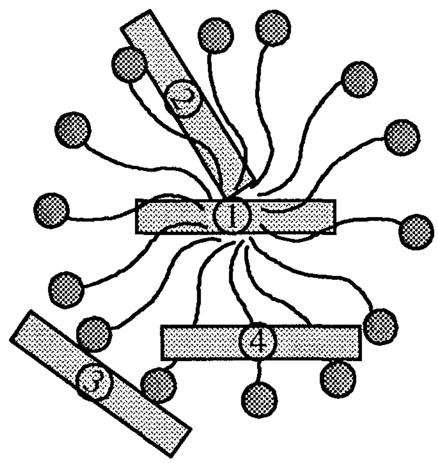


Figure 2.6 Sites within a micelle where solubilization occurs.

The amount of HOC that is solubilized will depend on the size and shape of the micelle, the concentration of the surfactant, the solubilizate structure, and the aggregation number. The aggregation number corresponds to the number of surfactant monomers that will associate, to form a micelle, given a certain concentration of surfactant is present. All of these factors depend primarily on the hydrophobic portion of the surfactant. If the hydrophobic chain of the surfactant is long then the CMC is smaller, the aggregation is larger, and the volume of the hydrophobic core is larger. A longer surfactant hydrophobic chain would allow more of the HOCs to be incorporated into the micelle, ultimately increasing the apparent aqueous solubility and bioavailibility of the compounds. However, as the chain length of a contaminant lengthens, the extent of solubilization appears to decrease independent of the surfactant structure (Rosen,1989:178). By utilizing a surfactant that provides a large hydrophobic core volume and a high aggregation number,

more of the HOC of interest can be made bioavailable by increasing its apparent solubility (Rosen,1989:179).

2.5 SURFACTANT ENHANCED BIOREMEDIATION

Laboratory research has shown that surfactant addition increases the mineralization rate of most hydrophobic compounds. Aronstein *et al.* (1991) studied the effects of low concentrations of nonionic surfactants on the biodegradation and desorption of sorbed aromatic compounds in soil, and found that the addition of surfactants improves the biodegradation rate of anthropogenic compounds. Sub-critical micelle concentrations of two nonionic alcohol ethoxylate surfactants, Alfonic 810-60 and Novell II 1412-56, were used. Low concentrations were used because inhibition of microbial metabolism at high concentrations was a concern due to surfactant toxicity. Phenanthrene and biphenyl were used as HOCs to contaminate two types of media. No detectable enhancement of the desorption rate of either HOC was noted when different concentrations of Novell II and Alfonic 810-60 were added to the soil slurries. However, an enhancement of the mineralization of phenanthrene was noted with the addition of Alfonic 810-60 at a concentration of 10 μg/g (μg of surfactant/g of soil) in both soils. At this surfactant concentration nearly 50% of the added phenanthrene was mineralized in 495 hours compared to only 4.8% in the absence of the surfactant. Similarly, Novell II stimulated the biodegradation of phenanthrene in both soils. However, at a higher concentration (100 μg/g, of Novell II), inhibition of the microbial populations was noted. Under similar test conditions for biphenyl, neither of the surfactants showed any significant enhancement of biodegradation in either of the two soil types. From their experiments, the authors

concluded that the addition of surfactant increased the extent of desorption, due to the lowering of the interfacial free energy and resulted in more substrate being made available to the microorganisms present to allow for more microorganism growth and mineralization (Aronstein *et al.*, 1991).

Aronstein and Alexander (1992) further studied the effects of sub-micelle concentrations of Alfonic 810-60 and Novell II 1412-56 on desorption and biodegradation of phenanthrene and biphenyl in batch assays using an aquifer sand (.4% organic matter). Both surfactants significantly enhanced the desorption of the HOCs at surfactant concentrations of 10 and 100 μg/g, and also improved the biodegradation of phenanthrene by the indigenous microbiota over surfactant-free controls. Enhanced partitioning of the HOCs to the aqueous phase due to the influence of the surfactants was suggested as reason for enhancements in biodegradation. To investigate the effects of surfactant losses due to sorption, Aronstein and Alexander conducted soil column assays with surface application of surfactant solutions. A soil column assay allows for a mass balance to be accomplished on the amount of surfactant and HOC that is present in the soil system. In these studies low levels of Novell II 1412-56 enhanced mineralization of both phenanthrene and biphenyl in a mineral soil (7.6% organic matter). Applied surfactant concentrations of 100 μg/g were less effective than lower concentrations of 10 μg/g, probably due to inhibition of the organisms due to toxicity. Further analysis indicated that neither HOC was being lost due to volatilization and that the low levels of applied surfactants had no effect on leaching of contaminants. The authors concluded that several factors influence the effectiveness of a surfactant, including: the contaminant of concern,

characteristics of the soil type as shown by the differing effects on biodegradation of the two soil types, and the concentration of surfactant addition (Aronstein and Alexander, 1992).

Bury and Miller (1993) investigated the effect of micellar solubilization on biodegradation rates of *n*-decane and *n*-tetradecane by addition of Neodol, a nonionic ethoxylated alcohol (distributions from C_{12} to C_{15}) surfactant, and two pure cultures of gram negative bacteria (Pseudomonas aeuroginsa and Ochbactrum anthropi). As the concentration of the Neodol surfactants was increased, the aqueous concentration density of *n*-decane and *n*-tetradecane was found to increase linearly. Similarly, the addition of surfactant also increased the mineralization of both HOCs, with reductions in cell density doubling times when compared to controls without surfactant. The Monod model was assumed to apply, and the rate of hydrocarbon consumption (dS/dt) was equated to the rate of cell growth (dX/dt) as follows (Bury and Miller, 1993):

$$\frac{dS}{dt} = -\frac{dX/dt}{Y} = -\frac{\mu_{max} S}{Y(K_s + S)} X \qquad (2\text{-}2)$$

where X = cell density

S = concentration of substrate

μ_{max}= maximum growth rate of microorganisms

K_s = half saturation constant

Y = a yield coefficient (mg of dry cells/mg of hydrocarbon oxidized)

Y = a yield coefficient (mg of dry cells/mg of hydrocarbon oxidized)

At low substrate (HOC) concentrations ($S \ll K_s$), increasing the concentration of the available substrate, due to surfactant solubilization, results in increased microbial growth. However, at high substrate concentrations ($S \gg K_s$), increasing the concentration of available substrate has no significant impact on microbial growth. Experiments were conducted to show that hydrocarbon losses to volatilization (evaporation) were negligible, thus verifying the model assumption that the HOCs were oxidized by the microorganisms. With this model, cell growth and hydrocarbon consumption were predicted for the batch reactors, and were used as a basis to conclude that the main factor contributing to the enhancement in mineralization was solubilization (Bury and Miller, 1993).

Thibault *et al.* (1996) investigated the influence of four surfactants at five concentrations on their ability to desorb and degrade pyrene in soils. Two composite samples of pyrene, a polycyclic aromatic hydrocarbon (PAH), contaminated soil from different sites were used for batch reactor analysis. The four nonionic surfactants used were Simple Green, Biosolve, Witconol SN70, and sodium dodecyl sulfate (SDS). Both unsaturated and saturated conditions were investigated in this study. The tests revealed that all of the surfactants increased the mineralization of the pyrene under unsaturated conditions, with the effectiveness of the surfactants being Witconol SN70>SDS>Biosolve>Simple Green. However, under saturated conditions the surfactants inhibited the microbial activity, and the amount of mineralization without surfactant addition was greater than with surfactant addition. The study by Thibault revealed that inoculation of pyrene degraders in the

presence of surfactant resulted in an increase in the amount of pyrene that was degraded in unsaturated conditions. The higher rate of mineralization in the saturated soil slurries (without surfactant addition) can be attributed to the microorganisms having more water available for growth, plus the pyrene having higher aqueous concentration to allow for greater bioavailability with the microorganisms, thus increasing mineralization (Thibault *et al.*,1996).

At one installation in California, approximately 60,000 gallons of No. 2 diesel fuel leaked from an underground tank and contaminated soil from 6 to 34 meters below the surface. Treatability studies were conducted to determine if surfactant flooding was a viable alternative (Peters *et al.*, 1992), and the only reference which was found to relate to an actual field site. A total of 22 surfactants were screened to determine which were the most effective at solubilizing and mobilizing the hydrophobic organic compounds of the diesel fuel to enhance biodegradation. Soil samples from the site were collected from soil borings. Gas chromatography analysis was used on the supernatants which were centrifuged from each of the soil slurries prepared. The results of the total petroleum hydrocarbons analysis showed that the anionic surfactants provided for the greatest amount of solubilization. Based on the laboratory analysis, the three surfactants which performed the best were Surfynol 485 (nonionic), Cyanamer P-35 (anionic), and Poly Sodium vinyl Sulfonate (anionic). Each of these surfactants showed enhancement of biodegradation close to 90%, when compared to treatments without surfactant addition. The authors concluded that two techniques could be applied in the field using the surfactants which resulted in the highest mineralization of the diesel fuel. The two systems

would be either *in situ* or on-site. The *in situ* system would involve injecting surfactant into the contaminated zone, and circulating the surfactants to provide for mobilization of the diesel fuel components. The on-site system would involve excavating the contaminated soil and transferring it to a slurry reactor where surfactant would be added, and the mixture would be agitated. The sludge from the reactor would then be processed through a biological wastewater treatment system. Overall, the addition of surfactant was anticipated to increase the mineralization of the organic components in the diesel fuel.

2.6 LITERATURE REVIEW SUMMARY

In recent years, attention has been given to enhancing *in situ* bioremediation. The addition of surfactants allows for the increased mobility of hydrophobic organic compounds through solubilization and wetting of interfaces. Solubilization and mobility are directly related. As more of a contaminant becomes soluble in water, more of the contaminant is available to move freely (mobile). Surfactants decrease the surface tension of the aqueous system which allows for the increased mobility. Studies by Alexander and Aronstein, Bury and Miller, Thibault *et al.*, and Lewis show that the degradation of various organic compounds can be enhanced by addition of surfactants of the nonionic type. Surfactants can be used in *in situ* remediation projects by modifying pump and treat systems to introduce surfactant. Through addition of surfactants in pumping systems (soil flooding), the availability of hydrophobic organic compounds to microorganisms can be increased, thus providing for population growth and increased mineralization of the contaminant. Each contaminated site requires that the following factors be taken into account: type of surfactant to be used for contaminant present, type of soil on site, amount of substrate

(contaminant), and the concentration of surfactant that should be used. Each of these items can drastically affect any remediation design involving the addition of surfactants. If the wrong surfactant is chosen or too much is used, inhibition of the microbial populations can result. In conclusion, from the literature presented, surfactants, when properly applied, may enhance the biodegradation of many hydrophobic organic compounds.

3. Methodology

3.1 OVERVIEW

This methodology describes how this study was accomplished to show that an enhancement or inhibition of microbial metabolism was achieved, through addition of surfactants. The experimental setup utilized a respirometer to measure the metabolism of indigenous soil microbes, based on the amount of oxygen consumed and carbon dioxide evolved. Soil microcosms were prepared and artificially contaminated with JP-8 fuel to simulate a spill in the vadose zone of an aquifer. Surfactants were added to several microcosms to determine if synergistic or antagonistic (enhanced or inhibited bioremediation) results would occur. The microcosms were connected to the respirometer which allowed for monitoring of the headspace gases over a 21 day period. Each microcosm was sampled at regular intervals, typically every 6 hours, over the period. The data collected by the respirometer allowed for evaluating the biological activity of soils contaminated with JP-8, soils amended with surfactants, and soils contaminated with JP-8 and amended with surfactants. Based on the increase or decrease in the oxygen consumption/carbon dioxide evolution observed, enhancement, inhibition, or no change of microbial metabolism could be concluded.

3.2 SOIL SELECTION

Baker (1995) and Totten (1995) used three types of soil to evaluate the degradation of JP-8 fuel, and found that all three soils provided for degradation of the fuel. For this study only one type of soil was used, it was from the same source as "Soil A" as used by Baker

(1995). This soil was chosen because it has a moderate organic matter content which provided for sorption of the contaminant. As seen in Chapter 2, sorption causes slow biodegradation rates. The properties which control the sorption of a contaminant are its solubility and hydrophobicity due to non-bioavailability. The hydrophobic nature of a contaminant results in a strong bond forming between the soil's organic matter and the contaminant. The contaminant sorbs in the micropores of the soil, where microorganisms can not access it. Therefore, by using a soil with a moderate organic content, surfactant addition was evaluated to determine if desorption and solubilization of the contaminant from the micropores of the soil will increase bioavailability and biodegradation.

3.2.1 Soil Collection

The soil used in this study, Kittyhawk silt, was collected from a wooded area on Wright Patterson AFB, OH. As mentioned earlier, the Kittyhawk silt soil is identical to "Soil A" as used by Baker (1995). The area where the soil was collected comprised an undisturbed area adjacent to a creek bed. The soil was rich and dark, and appeared to be of a clay nature, since it rolled into balls without water addition.

The procedures for collecting and processing the soil were identical throughout all experiments conducted. All samples were collected from 12 to 24 inches below the surface, using a clean steel shovel. The soil was placed in a plastic bucket lined with a plastic bag to present cross contamination. After collection, the soil was sieved using a 6mm sieve to remove stones, leaves, roots, and other foreign matter. The soil was then

placed in a large plastic tub (1m x 1.5m x .3m) where it was thoroughly mixed. After mixing the soils, 100 grams of the Kittyhawk silt was weighed using a OHAUS Harvard Triple balance, and placed in 250ml Pyrex bottles which were used as microcosms during the experiments.

3.3 RESPIROMETER

The respirometer used in this study was a Micro-Oxymax respirometer, as manufactured by Columbus Instruments International Corporation, Columbus, OH. The Micro-Oxymax respirometer, a closed circuit respirometer, was used to measure minute amounts of oxygen consumed and carbon dioxide evolved by a sample. Refer to Figure 3.1 for a picture of the respirometer. A computer functioned as a system controller and data collection device for the respirometer. The respirometer used two gas sensors to measure changes in oxygen and carbon dioxide concentrations in the headspace of each microcosm connected to the system. A system sampling pump circulated air from each microcosm through the oxygen and carbon dioxide sensors and returned the air back to each microcosm. The system used in this study was capable of monitoring 20 microcosms. It used a combination of two interface devices to monitor the microcosms. The interfaces provided for individual monitoring of each microcosm through input and output manifolds, with normally closed micro valves that controlled the airflow through each microcosm. The respirometer was capable of refreshing the air in each chamber or microcosm. This was done to ensure adequate oxygen levels were maintained to support microbial activity, and mainly to ensure the concentration of gases remained within the

detection limits of the sensors. The oxygen sensor used in this study had a range of 19.3% to 21%, and the carbon dioxide sensor had a range of 0% to 1%. The oxygen sensor used in this system was an electrochemical device, similar to a fuel cell, and measured oxygen percentage directly. The carbon dioxide sensor utilized was a single beam, nondispersive infrared device. By measuring the changes in gas concentrations over time, the amount of degradation was determined for a contaminant. For a more detailed discussion on the operation and theory of the Micro-Oxymax refer to Baker (1995).

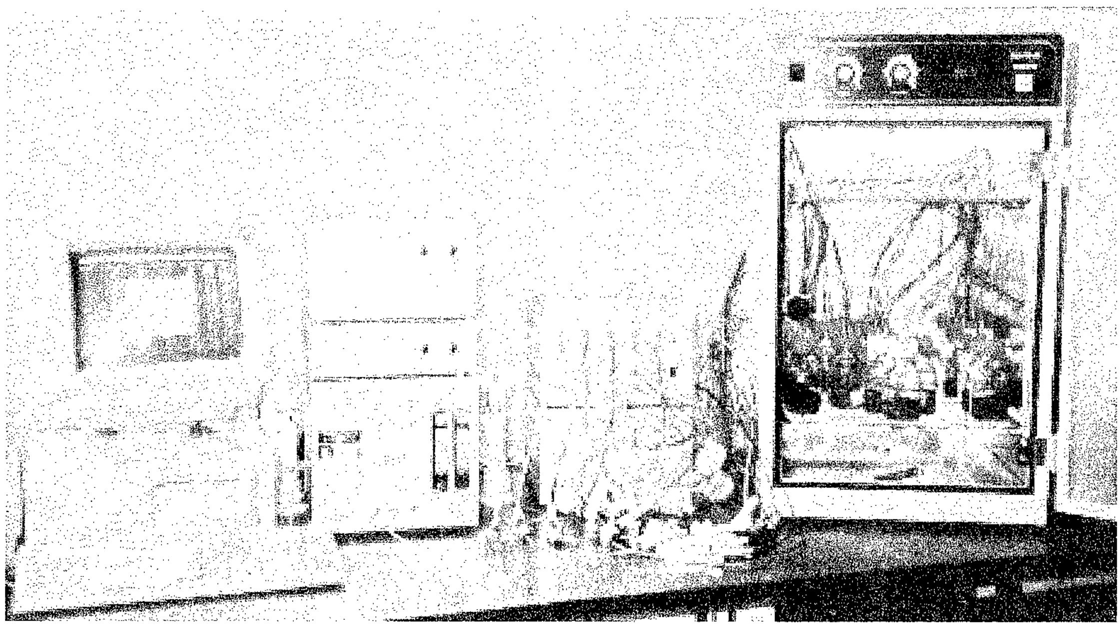


Figure 3.1 Picture depicting the Micro-Oxymax respirometer, and the Lab Line incubator used for temperature control. The CPU on the left serves as the system controller; to the right of the CPU is the carbon dioxide sensor, oxygen sensor, and system sampling pump (from top to bottom); adjacent to the sensors and pump are the two interface devices which connect to the 20 microcosm bottles in the Lab Line incubator (on the far right) by 1/8"nylon tubing.

3.4 Sample Preparation

3.4.1 Soil Characteristics

The characteristics of the Kittyhawk silt were important for determining the availability of contaminants when a spill occurs. A soil particle analysis was previously accomplished in the studies by Baker (1995), in accordance with ASTM Method D-422. The results of this analysis appear in Table 3-1.

Table 3-1 Soil Particle Analysis

SOIL	PARTICLE SIZE ANALYSIS * (%)						GROUP NAME	GROUP SYMBOL	MOISTURE CONTENT
	Gravel	Coarse Sand	Medium Sand	Fine Sand	Silt	Clay			
Kittyhawk silt	3	5	13	24	39	16	Sandy silt	ML	25.3%

Source: Baker (1995)

The chemical composition of the soil was also important to determine if biodegradation could be implemented as a remedial alternative. The proper amount of nutrient sources (i.e. phosphate and nitrate) must be present to allow for microbial processes to be carried out. In addition to the physical analysis above, a chemical analysis was also done to determine the pH, organic carbon content, nitrates, ammonia, and phosphates present. A summary of this data can be found in Table 3-2.

Table 3-2 CHEMICAL ANALYSIS OF SOIL					
SOIL	**pH**	**ORGANIC CARBON**	**NITRATES**	**AMMONIA**	**PHOSPHATES**
Kittyhawk silt	7.92	7.04	280	20.3	2.80

Source: Baker (1995)

From the physical and chemical analysis, the Kittyhawk soil had adequate organic content to provide for sorption of contaminants. Also, the soil had adequate nutrients to support biodegradation.

3.4.2 Soil Moisture

Water is essential for microbial maintenance and growth. It serves as a transport medium for bacteria, nutrients, and contaminants. The lack of water can be detrimental to microbial sustenance in the vadose zone. Too much water fills the pore spaces, and limits the amount of oxygen available to support aerobic activity of the microbes. In general, biodegradation of contaminants in soil systems is optimal at a moisture content of 30% to 80% of the field capacity (Dibble and Bartha, 1979; Riser-Roberts, 1992). Field capacity represents the water holding capacity of a soil, and is the amount of water remaining in the soil after adequate drainage as a result of gravitational (capillary) forces (Baker and Herson, 1994: 212). Most soil microbiologists utilize a moisture content of 50% to 60% of field capacity, to maintain optimal conditions for microbial metabolism (Baker and Herson, 1994: 212). The water content of the microcosms in this study could impact the metabolism of the microbes present which would ultimately affect the amount of JP-8 that

can be transformed. Therefore, the field capacity of the soil used in this study was determined to ensure adequate moisture content, using the estimation method as presented by Baker and Herson (1994:212).

A moisture content of 60% of field capacity was chosen as the level of water content for this study. This level was chosen since it provides the optimum conditions for biodegradation, and ensured adequate water content in the microcosms throughout the study. Distilled water was added to each microcosm to adjust the natural soil water content to the 60% of field capacity level. By adjusting each microcosm to 60% of field capacity ensures that each microcosm had the same amount of water for microbial metabolism, and limited the amount of variation in biological activity.

3.5 EXPERIMENT SET-UP

The physical setup for each experiment was identical. Figure 3.2 provides a schematic diagram for the experimental setup. The 250 ml microcosm bottles were connected to their respective monitoring units by 1/8"outside diameter nylon tubing. Moisture and organic vapor filters were placed in line, allowing for a mass balance on the carbon volatilized, and preventing damage to the sensors in the system. Moisture filters were placed in line before the carbon filters to prevent corrosion of system components, and to prevent absorption of the water vapor on the carbon filter. The microcosms were placed in a AMBI-HI-LOW incubating chamber, as manufactured by Lab Line, to control temperature variations and eliminate light.

Prior to beginning each experiment, the system sensors, tubing, and all microcosms were leak checked, using the automated leak check provided with the respirometer software. The automated leak check pressurizes the system components and measures changes in volume down to 0.001 μl. Also, the gas sensors were calibrated to ensure precise measurements. Nitrogen was first circulated through the sensors to obtain a zero reading for the carbon dioxide sensor. Then a primary standard calibration gas was passed through the sensors to calibrate the sensors to the known concentrations of oxygen and carbon dioxide in the standard. The primary standard used was from Liquid Carbonic Company, and contained 0.501% carbon dioxide and 20.4% oxygen, as reported on the cylinder. With all the tests performed and the sensors calibrated, the data collection could begin.

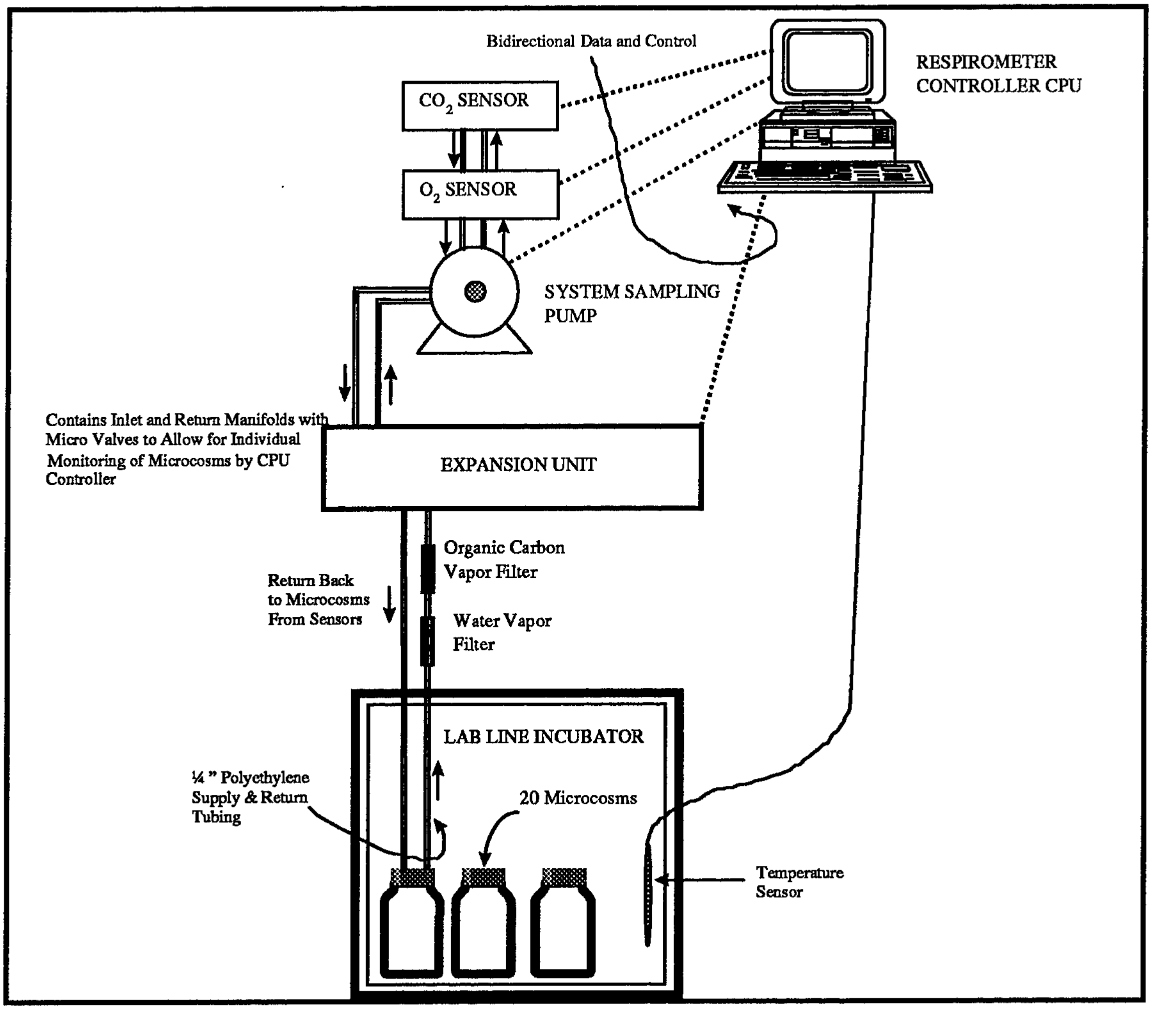


Figure 3.2 Schematic diagram of the experimental setup.

3.5.1 Organic Vapor Collection

Organic vapors can damage the oxygen and carbon dioxide sensors of the respirometer, and must be collected to prevent such damage. Also, collecting the organic vapors will provide an estimate on the amount of JP-8 lost to volatilization. Volatilization has been considered one of the primary pathways for removal of volatile contaminates from the vadose zone (Dean-Ross et al, 1992). As such, it must be taken into account when considering bioremediation studies, to ensure that biodegradation has taken place. To collect organic vapors in this study, organic vapor collection tubes were prepared. The

tubes used in this study were made of Pyrex glass, with a .6 cm inside diameter and approximately 8 cm long. The tubes were filled with granular activated carbon, and the tubes were sealed at either end with rubber stoppers with 1/4" holes. The granular activated carbon was ground using a mortar and pestle to increase the amount of carbon that could be placed in the tubes. The carbon filters were then placed in line with each microcosm's supply line, to the system sensors.

3.5.2 Moisture Removal

With the microcosms being adjusted to 60% of field capacity, there was a considerable amount of water present in the headspace as vapor. Water vapor could interfere with the measurement of carbon dioxide levels, and could cause corrosion within the respirometer. The Micro-Oxymax was provided with two separate drier systems; one was for outside air filtration during refreshing, and the second was used prior to the gas sensors. The air drier for refreshing consisted of a packed column of Drierite, which changes color from blue to red, indicating the presence of water. The air drier system, for the sensor loop, consisted of two redundant driers which could be alternated to allow absorbent to be changed in the drier not being used. These drier columns used magnesium perchlorate as an absorbent, with cobalt chloride as an indicator.

Since a mass balance was being conducted using carbon vapor traps, moisture present in the headspace could affect the absorbed mass of the carbon. To eliminate this problem individual moisture filter tubes were placed in each microcosm's supply line prior to the

carbon filter. The tubes were identical to those used for the organic vapor filters, as described above. The absorbent used in the tubes was magnesium perchlorate, with trace amounts of cobalt chloride as an indicator. The ends of the tube were sealed with rubber stoppers with 1/8" holes, for connection to the microcosms by nylon tubing. The tubes were replaced every 3 to 4 days as the maximum absorptive capacity was reached.

3.5.3 Temperature Control

During preliminary studies, it was determined that temperature control was an important parameter to consider for experimental design. The metabolic activity of microorganisms could be influenced by fluctuations in temperature. As the temperature increases, metabolic activity increases; likewise as the temperature decreases, metabolic activity decreases. During the preliminary studies with the respirometer, the microcosms were left out in ambient room conditions. The room temperatures fluctuated from 3° to 5° C, and resulted in cyclic uptake rates which fluctuated from 100 μl to 250 μl/hr. Refer to Figure 3.3 for an example of the temperature fluctuation effect on oxygen uptake for indigenous soil microflora. Since small temperature fluctuations resulted in cyclic rates, the microcosms were placed in a AMBI-HI-LOW incubating chamber, as manufactured by Lab Line, to keep temperature fluctuations to a minimum. The incubator's thermostat was set to 30° C, to allow for proper temperature control, due to lack of ambient temperature control within the laboratory.

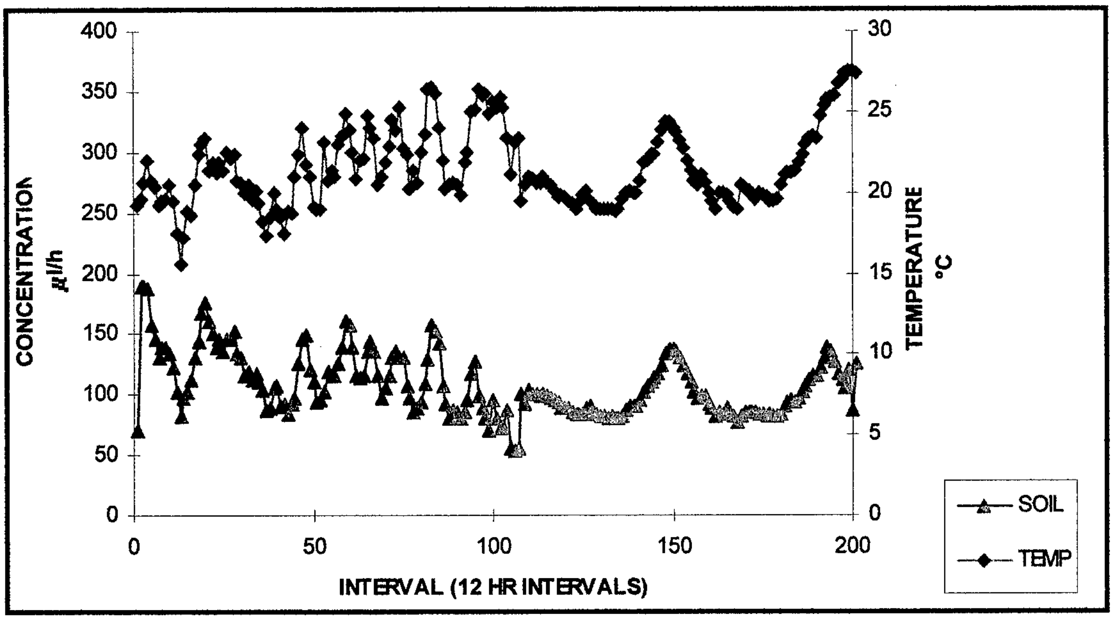


Figure 3.3 Effect of temperature on the respiration of soil microorganisms. Graph of the oxygen consumption rate of uncontaminated Kittyhawk silt soil. Small fluctuations in Temperature result in increases or decreases in the respiration rate of the indigenous organisms present in the soil. Each observation (interval) represents a 12 hour time period from the previous interval.

To accommodate the microcosm tubing connections, two 2 ½ inch holes were cut in the back of the incubator to connect the tubing to the microcosms. The incubator was sealed to exclude light from inducing activity of any photo autotrophic organisms which might be present in the soil samples.

3.6 LABORATORY PROCEDURES

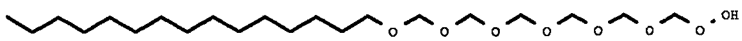
3.6.1 Surfactant Addition

To determine the effects of surfactant addition, two surfactants were chosen based on the research, described in the literature review, of Aronstein *et al.* (1991). The two surfactants used in this study were Novell II 1412-7 and Alfonic 810-4.5. Both of the

Chemical Company. The surfactants were added at three concentration levels (sub - CMC, CMC, and supra-CMC), to determine the effect of surfactant concentration on microbial metabolism, and the potential for biodegradation enhancement. Since Aronstein *et al.* (1991) and Edwards et al (1991;1992) both found enhancements using sub-CMC surfactant concentrations and CMC concentrations, two of the treatments were chosen such that the concentrations applied were at these levels also. The third level of surfactant concentration chosen was a supra-CMC concentration to determine if further enhancement would be seen. Each level of surfactant concentration was separated by a factor of 10. The CMC concentrations of the Novell II and Alfonic surfactants were reported by the manufacturer as 3.8 and 172.1 ppm respectively. Refer to Table 3-3 for a summary of the surfactant properties.

The partitioning coefficient (K_D) was estimated for each surfactant to take into account adsorption of surfactant on to soil organic matter. The K_D was estimated at 0.48 and 0.80 for Novell II and Alfonic surfactants respectively. The K_D estimates were obtained from similar ethoxylate nonionic surfactants, with similar molecular weights.

Table 3-3 Properties of surfactants used for this study.

Surfactant/ Surfactant Structure	Avg MW	CMC (ppm)
Alfonic 810-4.5 	378.25	172.1
Novell II 1012-7 	518.74	3.8

Source: Vista Chemical Company

A stock solution of each surfactant was made to add to the individual microcosms. Surfactant solutions were mixed in 200 ml flasks. The appropriate amount of surfactant was added to each flask, by weighing the surfactant added with an OHAUS electronic balance. After the surfactants were added to each flask, distilled water was added, to fill each flask to the 200 ml volumetric fill mark. The solutions were placed on a stirring plate for 2 hours to ensure adequate mixing. After stirring, the surfactant solutions were added to each microcosm using a graduated pipette. Refer to Table 3-4 for a summary of the surfactant concentrations added. After the surfactant solutions were added, distilled water was added to bring the microcosm up to 60% field capacity. Clean spatulas were used to mix the soils thoroughly.

Table 3-4 Surfactant Levels of Treatment. Each microcosm has 100 g of soil, of which 20 ml is water present. Each microcosm had 10 ml of water/surfactant added

SURFACTANT	Novell II			Alfonic		
LEVEL	Sub CMC	CMC	Supra CMC	Sub CMC	CMC	Supra CMC
Estimated K_D	0.48			0.8		
CMC Given mg/l	3.8			172.1		
Concentration of Surfactant Stock mg/l	295			9300		
Amount of Distilled Water Added ml	9.95	9.5	8	9.75	7.5	0
Amount of Surfactant Added ml	0.05	0.5	2	0.25	2.5	10
Concentration of Surfactant Dose mg/l	0.492	4.92	49.2	31	310	3100

3.6.2 Soil Contamination

Select microcosms of Kittyhawk silt were contaminated with JP-8 fuel. The fuel sample was provided by the United States Air Force Fuels Research Laboratory, Wright Patterson AFB, OH. Baker (1995) had used two different levels, 0.1% and 1.0% (by weight), of JP-8 in respirometry studies previously conducted. The focus of this study required only one level of JP-8 contamination to be used. Prior to selecting the level of contamination, preliminary respirometer analysis was conducted using a 0.1% (by weight) level of JP-8 contamination. This study was conducted to ensure that a statistically significant difference in respiration was achieved between the contaminated and uncontaminated Kittyhawk silt soil.

Since the microcosms contained 100 g of soil each, a 0.1% level of JP-8 corresponds to 100 mg. The JP-8 was weighed with a digital mass balance to determine the correct amount of fuel to add volumetrically to each microcosm. A 0.05 ml graduated pipette was used to add JP-8 to a 10 ml beaker on the digital scale. When 100 mg registered on the scale, the volume of JP-8 added was annotated. This was accomplished three times, and an average of the volumes added was used as the volume of JP-8 to add. Table 3-5 summarizes the results of the data.

Table 3-5 Determination of JP-8 Volume to be Added

Trial Number	1	2	3	**Average**
Volume of JP-8 Added (ml)	.16	.15	.14	**.15**

The results of the preliminary study (Figure 3.4) indicated that a 0.1% level of JP-8 contamination provided for adequate delineation from background soil respiration.

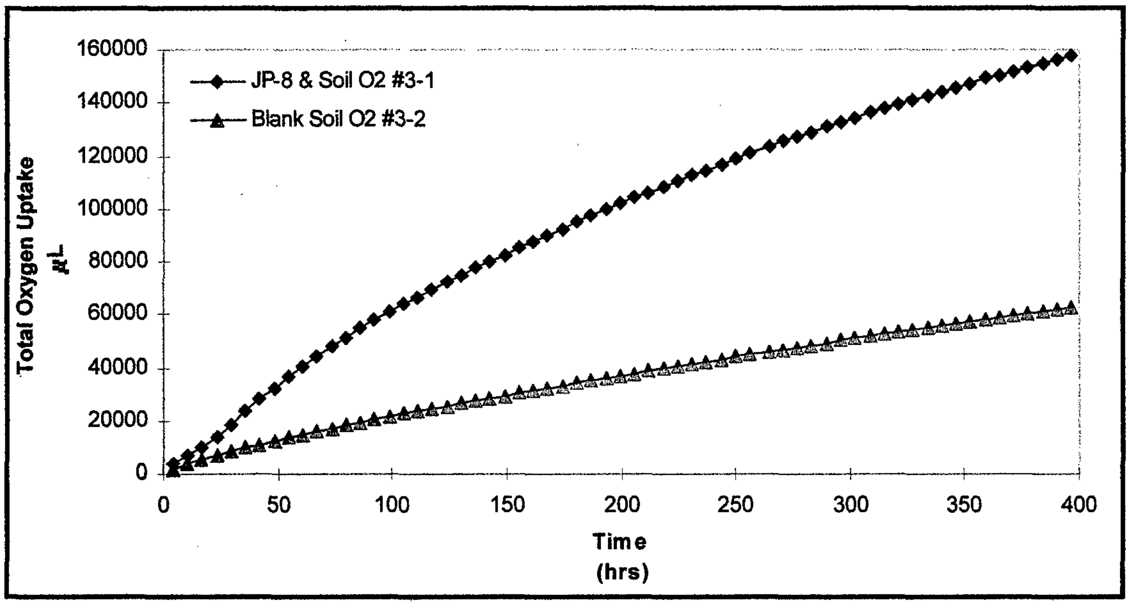


Figure 3.4 Preliminary Study of JP-8 *In Situ* Biodegradation.
This graph compares the respiration of uncontaminated and contaminated Kittyhawk silt soil. A 0.1% level of JP-8 was used to determine if a statistically significant different oxygen uptake would occur for the contaminated and uncontaminated soil microcosms.

3.6.2.1 JP-8 Addition to Microcosms

JP-8 was added to each microcosm, as the last additive, to ensure that volatilization was kept to a minimum. The JP-8 was added to the microcosms after surfactant and distilled water had been added, and adequately mixed. JP-8 was added using a 0.05 ml graduated pipette, a total of .15 ml was added to each treatment requiring the JP-8 to be added. After the JP-8 was added, the soil was mixed with a clean spatula to ensure adequate mixing, and the microcosm was then sealed and connected to the respirometer.

3.7 DATA COLLECTION

The Micro-Oxymax respirometer recorded the amount of oxygen and carbon dioxide gases (by percent) present in each microcosm, every six hours. Using this data, the controller for the respirometer then calculated and recorded the following parameters: % oxygen, % carbon dioxide, oxygen consumption rate ($\mu l/min$), carbon dioxide evolution rate ($\mu l/min$), cumulative oxygen consumption (μl), cumulative carbon dioxide evolution (μl), temperature (°C), and the respiratory exchange rate (RER—the ratio of carbon dioxide production to oxygen consumption).

3.8 DATA ANALYSIS

The techniques used to perform the data analysis involved graphical comparisons, plus descriptive and analytical statistics. The biological activity of the indigenous soil microorganisms was compared for uncontaminated, JP-8 contaminated, surfactant amended, and JP-8 contaminated with surfactant amended soil microcosms. The focus of the data analysis was to determine the following:

- Reproducibility of the Micro-Oxymax Respirometer.
- If biodegradation of JP-8 occurred.
- Given that JP-8 degradation occurred, do surfactants enhance the biodegradation of JP-8.

3.8.1 Respirometer Reproducibility and Repeatability

The reproducibility of the respirometer data between experiments was critical to limit experimental error. Experimental error referred to fluctuations in replicate observations from one experiment to another. It refers to variation that is unavoidable. Reproducibility and repeatability describe the precision of experimental data. Repeatability refers to precision of replicates within the same experimental run or interval, whereas, reproducibility refers to precision between replicates of different experimental runs. According to Berthouex and Brown (1994:12), the between-run precision has a greater spread than within-run precision. Therefore by examining both reproducibility and repeatability of the Micro-Oxymax respirometer, the total variability in the measurement process would be determined.

Replicates for each treatment were compared graphically, for comparison of total oxygen uptake over time. An estimate for the standard deviation (standard error), of each sampling interval, was also examined. The standard error ensured that the data obtained by the respirometer is precise within experimental runs. From the standard error at each sampling interval, a determination was made about the repeatability of the respirometer.

3.8.2 JP-8 Degradation

Prior to determining if surfactant enhancements have occurred, JP-8 biodegradation was demonstrated. To ensure that the JP-8 was degrading, the respiration of uncontaminated and JP-8 contaminated Kittyhawk silt soil was compared using a statistical hypothesis test

procedure. According to Devore (1995), a statistical hypothesis is a claim about the value of a single population characteristic, or about the values of several characteristics. A two tail independent t test was used with a 0.05 level of significance. The objective of the test will be to determine which hypothesis was correct. The null hypothesis, H_0, was that no biodegradation of JP-8 took place, and the sample mean oxygen consumption for the uncontaminated ($\bar{x}_{UNC}$) and JP-8 contaminated ($\bar{x}_{JP8}$) soil microcosms were equal. The alternate hypothesis, H_a, was that biodegradation had occurred, and the sample mean oxygen consumption for the JP-8 contaminated soil microcosms was greater than the uncontaminated microcosms; the second alternate hypothesis was that inhibition of the microorganisms had occurred, and the mean oxygen consumption for the JP-8 contaminated soil microcosms was less than the uncontaminated microcosms.

3.8.3 Surfactant Enhanced JP-8 Degradation

The determination of surfactant enhanced biodegradation was fairly straightforward when measuring the amount of respiration for indigenous soil microbes. For this study, enhancement was defined as an increase in the amount of oxygen uptake (measured in μl), in the contaminated soil microcosms with surfactant addition. The increase must have been greater than the combined oxygen uptakes for the individual treatments, for surfactant and JP-8 contaminated microcosms, to conclude that enhancement had occurred. A statistically designed hypothesis test was used to compare the oxygen uptake of soil microcosms that were uncontaminated, contaminated with JP-8 fuel, surfactant

amended, and contaminated with JP-8 and surfactant amendment. A critical t value approach was used with a 0.05 level of significance. The null hypothesis, H_0, was that there was no effect on the oxygen consumption from surfactant addition, and $\overline{X}_{DIFF}$ was equal to zero. There were two alternate hypothesis that were conducted to determine if inhibition or enhancement had occurred. One alternate hypothesis, H_a, determined if a statistically significant increase in oxygen uptake, enhancement, had occurred, compared to the null distribution. The other alternate hypothesis determined if a statistically significant decrease in the oxygen uptake, inhibition, had occurred, compared to the null distribution.

3.9 STATISTICAL EXPERIMENTAL DESIGN

The experimental design was established to allow for statistical hypothesis tests to have been completed about the sample means. To evaluate variation in sample measurements, three replicates for each treatment were determined as the minimum optimal design parameters. Since there was 16 separate treatments that had to be evaluated, and the Micro-Oxymax could only monitor 20 microcosms at once, a total of 3 experimental replications (runs) would have to be accomplished. The overall original experimental design matrix was as shown in Table 3-6.

Table 3-6 Experimental Design. This matrix design does not include replicates for blank microcosms which must be used as a blank sample to ensure the respirometer is operating correctly. One blank sample is required for each experimental run.

MICROCOSM EXP DESIGN WITH 100 g OF SOIL Number of Replications								
CONTAMINANT	SURFACTANT	No Surfactant	Novell Sub-CMC	Novell CMC	Novell Above-CMC	Alfonic Sub-CMC	Alfonic CMC	Alfonic Above-CMC
No JP-8		3	3	3	3	3	3	3
0.1% JP-8		3	3	3	3	3	3	3

The first experimental run was set-up as indicated in Table 3-7.

Table 3-7 Experiment #1 Setup

MICROCOSM ASSIGNMENTS FOR EXP #1								
CONTAMINANT	SURFACTANT	No Surfactant	Novell Sub-CMC	Novell CMC	Novell Above-CMC	Alfonic Sub-CMC	Alfonic CMC	Alfonic Above-CMC
No JP-8		2,17	4	5	6	7	8	9
0.1% JP-8		3,17	10,18	11	12	13,19	14	15
Empty Microcosms:1								

The system sampling pump, and one of the interfaces developed a leak, 30 samples into the experiment, and the data had to be rejected due to the system sampling pump failure. A second experiment was setup to continue the experimental design started. However, only 10 chambers could be sampled. Since Aronstein *et al.* (1991) had previously seen that concentrations slightly above the CMC Novell II concentration were slightly

inhibitory, the supra-CMC concentrations of Novell II were not tested. Also, the sub-CMC concentrations of both surfactants were not tested, since only 10 samples would be monitored. Table 3-8 contains a summary of the treatment design for this experiment.

Table 3-8 Experiment #2 Setup

MICROCOSM ASSIGNMENTS FOR EXP #2								
CONTAMINANT	SURFACTANT	No Surfactant	Novell Sub-CMC	Novell CMC	Novell Above-CMC	Alfonic Sub-CMC	Alfonic CMC	Alfonic Above-CMC
No JP-8		2		4			5	8
0.1% JP-8		3		6			7	9
Empty Microcosms:1								

This experimental setup revealed that inadequate mixing of contaminants was a possible problem due to different respiration rates of replicate microcosms. Also, the experiment revealed that Novell II appeared to be inhibitory, since the oxygen uptake curve for combined surfactant and JP-8 treatments was less than the JP-8 contaminated soil oxygen uptake curve. As a result, the experimental design was adjusted to eliminate Novell surfactant as a treatment objective. Eliminating the Novell treatment simplified the experimental design, and allowed for better replication of data, since more replicates would be accomplished per experimental run. Therefore, the experiment was modified to test only the three levels of treatment with Alfonic surfactant.

In experiment #3, for better mixing of contaminants and surfactants, the JP-8 was added first to the dry soils, and mixed thoroughly with a clean spatula. Surfactant was then

added, and the soil was again mixed with a clean spatula. The distilled water was the final additive to be added to the microcosms. The reason why JP-8 was not added first originally was to eliminate the possibility of vaporizing the contaminant as much as possible. However, it was determined that the focus of the study was not necessarily on the exact amount of contaminant present, but rather on the amount of enhancement seen, from measuring the oxygen uptake. By adding the JP-8 first and stirring the soils, each microcosm would essentially be treated the same. This made each microcosm subject to the same amount of agitation, and therefore the same amount of volatilization, due to mixing. The fact remained that it was not known exactly how much JP-8 was introduced, but since the same amount was added to each microcosm and each was subjected to the same mixing procedures, it was concluded that each microcosm received the same amount of contamination. This was verified by comparing oxygen uptake curves for the replicate treatments. Table 3-9 contains a summary of the microcosm treatment assignments for experiment #3.

Table 3-9 Experiment #3 Setup

MICROCOSM ASSIGNMENTS FOR EXP #3					
CONTAMINANT	SURFACTANT	No Surfactant	Alfonic Sub-CMC	Alfonic CMC	Alfonic Above-CMC
No JP-8		9,10	2,3,4	5,6	7,8
0.1% JP-8		11,12,13	14,15,16	17,18	19,20
Empty Microcosms:1					

With the conclusion of experiment #3, it was determined that the experiment would be completed one more time, to allow for contamination of the soil with JP-8 first, and left for three days prior to adding surfactants and water. This would allow for the JP-8 contaminated soils to adequately sorb any of the fuel hydrocarbon components, and attempted to simulate a period of acclimation, for the indigenous microbial populations present. Experiment #4 was a replicate of experiment #3, with this one exception. JP-8 was added and mixed with a clean spatula, and allowed to stand for a period of 72 hours. The surfactant and water were then added and mixed with a clean spatula also. The microcosms were then connected to the respirometer, and data collection was started. Table 3-10 depicts the experimental design for experiment #4.

Table 3-10 Experiment #4 Setup

MICROCOSM ASSIGNMENTS FOR EXP #4					
CONTAMINANT	SURFACTANT	No Surfactant	Alfonic Sub-CMC	Alfonic CMC	Alfonic Above-CMC
No JP-8		20	14,15	16,17	18,19
0.1% JP-8		2,3,4	5,6,7	8,9,10	11,12,13
Empty Microcosms:1					

Data obtained from Experiment #4 would be used for determining enhancement or inhibiting effects which resulted from surfactant addition.

4. DATA ANALYSIS

4.1 RESPIROMETER REPRODUCIBILITY and REPEATABILITY

By examining the cumulative oxygen uptake curves of identical treatments (refer to Appendix A for experiment 2-4 data results), within experiments and between experiments, the Micro-Oxymax respirometer appeared repeatable between replicates, within the same experiment, and somewhat reproducible between replicates of separate experiments. The results of experiments 2, 3, and 4 were each used to measure the reproducibility of the respirometer. Reproducibility was slightly less conclusive, due to inherent variability in the concentration of surfactant which was added to replicate samples, from one experiment to the next. The standard error plots, for the mean oxygen uptake, revealed that the error range was reasonably small for the majority of treatments, with exception of treatments with concentrations of CMC and supra-CMC Alfonic surfactant. There was a larger range of error for the CMC and supra-CMC Alfonic surfactant treatments since new stock solutions were made for the surfactant treatments, prior to the start of each experiment, which caused variability in oxygen uptake. This was primarily seen at the higher concentration surfactant treatments.

Since each of the experiments was conducted over slightly different time intervals, the oxygen uptake curves were examined over the time interval of 0 to 400 hours and 400 to 600 hours separately. Some variability in the oxygen consumption was expected since the diversity of microbial populations, in each microcosm, was a factor that could not be

controlled. This is perhaps the explanation for the slight variation in the oxygen consumption for uncontaminated Kittyhawk silt soil in Figure A.1. However, by examining the standard error of the mean oxygen uptake, for the uncontaminated soil samples, the amount of error was small at each sampling interval, which indicated that the respirometer was not only repeatable, but reproducible also. With the addition of other treatment factors, such as JP-8 contamination, surfactant addition, and JP-8 contamination with surfactant addition, more variability was introduced to the samples which would increase the amount of measurement error seen. The reason for the increase in measurement error was due to the lack of precision and accuracy from adding JP-8 and surfactant treatments to each microcosm. While each soil treatment was conducted identically, to limit variability to an extent, it was difficult to measure down to 0.05 ml accurately and precisely with a pipette. Examining Figure A.9, from 0 to 400 hours, the standard error for the JP-8 contaminated soil microcosms has a small error distribution, indicating reproducibility and repeatability. When CMC and supra-CMC concentrations of Alfonic surfactant were added, refer to Figures A.13 and A.15, there was a considerable amount of variation between experiments, which would indicate non-reproducibility. However, the treatments within experiments indicate that the respirometer was repeatable, since the oxygen consumption curves, within each experiment, overlie one another This indicates that measurement error, involved with adding surfactant treatments to soil microcosms, was caused by the variation in surfactant concentrations, rather than non-reproducibility of the respirometer. This same reasoning applies to the combined treatments of JP-8 and CMC/supra-CMC Alfonic treatments. However, it was noted that one of the oxygen consumption curves (#4-3) in experiment 4,

JP-8 with CMC Alfonic addition (refer to Figure A.13), exceeded the other within experiment replicates, and was concluded to have questionable results. Since this replicate exceeded the others (#4-1 and #4-2), it was determined to have questionable results, and it was concluded that something other than the surfactant treatment was causing the significant difference. After examining the suspect microcosm, at the end of the experimental run, small ants or termites were present in the soil, which was believed to have caused the significant difference in the oxygen uptake between the other two replicates. As a result this oxygen uptake curve (#4-3) was not used for further data analysis.

Results from the respirometer were concluded as being consistently repeatable, and reproducible depending on the amount of measurement error between replicates of separate experimental runs. The treatments for uncontaminated Kittyhawk silt soil and JP-8 contaminated soil, were accurate and precise over the time interval of 0 to 400 hours, while the surfactant treated soil microcosms were not as accurate or precise. Since the JP-8 came from the same sample for each treatment, the variation in treatments containing JP-8 was limited. However, since different surfactant stock solutions were used for each experimental run, variation was greater, and measurement error was introduced between experimental replicates. The reason the variation was more noticeable at the higher surfactant treatments was that the amount of carbon present for microbial metabolism increases as the concentration of surfactant increases. The oxygen uptake, required for microorganisms to assimilate carbon, increases or decreases with changes in the amount of carbon availability. The variability in carbon added to soil microcosms was seen as the

primary reason for variation in replicate treatments between experiments. Therefore it was concluded that the Micro-Oxymax respirometer was repeatable within experimental replicates, and was reproducible between replicates of separate experiments, if measurement error was kept to a minimum.

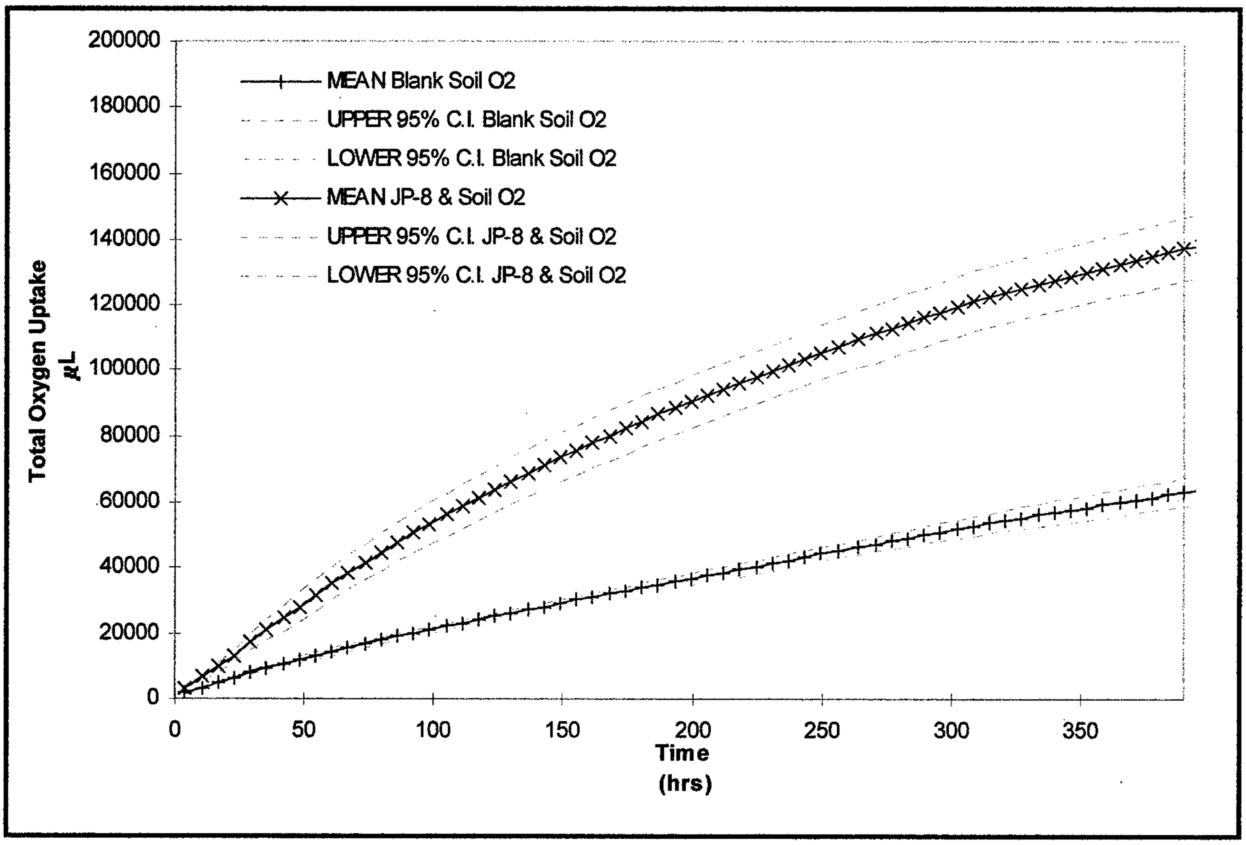
4.2 JP-8 DEGRADATION

The null hypothesis was that JP-8 fuel was not degraded, and had no effect on the metabolism of indigenous soil microorganisms. Biodegradation of JP-8 required that the null hypothesis be proven false. For biodegradation to be concluded, the difference of the two sample mean oxygen uptakes, for JP-8 contaminated and uncontaminated soil microcosms, must be significantly greater than the null hypothesis distribution which is centered at 0. A right tail t test was performed, with a 95% level of confidence, at each sample interval, to determine where biodegradation occurred. A summary of the sample data can be found in Table C-1 in Appendix C. At each of the intervals, the calculated t value was significantly greater than the critical t value. Therefore, the null hypothesis was rejected in favor of the alternate, and biodegradation was concluded at a 95% level of confidence. This implies that the probability is 0.95 that the null hypothesis interval does not include the true value of the mean difference, and that biodegradation of JP-8 occurred.

The mean cumulative oxygen uptake curves for JP-8 contaminated and uncontaminated soil microcosms are presented in Figure 4.1, along with their respective confidence intervals. From the data alone, it was apparent that the mean oxygen uptake curve for

JP-8 contaminated soil was significantly different.

Figure 4.1 JP-8 In Situ Biodegradation, JP-8 Contaminated Compared to Uncontaminated Kittyhawk silt Mean Cumulative Oxygen Uptake and 95% Confidence Intervals.



The oxygen uptake rate for the contaminated soil was significantly greater than uncontaminated soil. This was concluded based on the fact that the slope of the cumulative oxygen uptake curve, for JP-8 contaminated soil, was steeper than curve for the uncontaminated soil. Also, since the 95% confidence intervals for both means do not overlap, they were considered significantly different. Therefore, since biodegradation of JP-8 was concluded, the focus was now shifted to determine if the ultimate

of JP-8 contaminated soil would be enhanced, inhibited, or left unchanged as a result of surfactant addition.

4.3 SURFACTANT ENHANCED JP-8 DEGRADATION

The results from experiment #4 were used to determine the significance of whether there was no effect, inhibition, or enhancement of JP-8 biodegradation with surfactant addition at 3 levels of treatment. The null hypothesis was identical for each of three Alfonic surfactant concentrations treatments, and was based on the fact that there was no effect on JP-8 biodegradation. Each of the sample means were corrected for the natural background respiration of uncontaminated soil. Therefore, the difference of the corrected sample means, for JP-8 contaminated, surfactant amended, and JP-8 contaminated with surfactant amended soil microcosms, were compared to a null hypothesis that was centered around the difference being 0. One alternate hypothesis was that the addition of surfactants caused an inhibition of microbial metabolism, and resulted in less oxygen uptake - a smaller amount of JP-8 degradation. The confirmation of inhibition was accomplished by using a left tail t test. The other alternate hypothesis was that the biodegradation of JP-8 was enhanced from surfactant addition, and was validated with a right tail t test.

4.3.1 Sub-CMC Alfonic Surfactant Addition

A summary of the data can be found in Table B-1, in Appendix B. One of the combined treatment replicates was not used in the data analysis , because the chamber developed a leak in the middle of the experiment which resulted in the data collected by the respirometer, for the individual treatment, being invalid. From sampling interval 1 to 95 (0 to 600 hrs), the calculated t value fell within the 0.05 level of significance for the null

hypothesis. Therefore, the null hypothesis could not be rejected, and it was concluded that sub-CMC Alfonic addition had no effect on the biodegradation of JP-8, or the microbial metabolism of the indigenous soil microbes. Also it was noted that the 95% confidence interval included the means of all of the individual treatments considered. The primary reason for this was that only two replicates for the combined treatments of JP-8 and surfactant were measured, and resulted in the critical value for the t distribution being large (t_{crit} = 12.706) compared to the fact that using 3 samples results in a much smaller t value (t_{crit} = 4.303). This reduces the bounds of the interval by a factor of 3, and results in the bounds of the confidence interval including only the JP-8 and JP-8 with surfactant addition treatment means.

4.3.2 CMC Alfonic Surfactant Addition

A summary of the data can be found in Table B-2, in Appendix B. From sampling interval 1 to 82 (0 to 516 hrs), the calculated t value fell within the 95% confidence interval ($-2.447 \geq T \leq 2.447$) for the null hypothesis. Therefore, the null hypothesis could not be rejected. However from sampling interval 83 to 95 (516 to 600 hrs) the calculated t value was greater than the critical t value ($T \geq 2.447$) for the upper t tail test. Therefore it was concluded that, CMC Alfonic surfactant addition enhanced the biodegradation of JP-8 contaminated soil microcosms, with a 0.05 level of significance.

4.3.3 Supra-CMC Alfonic Surfactant Addition

A summary of the data can be found in Table B-3, in Appendix B. From sampling interval 1 to 60 (0 to 375 hrs), the calculated t value fell outside the 95% confidence interval for the null hypothesis, to the left of the lower tail t critical value ($T \leq 2.365$). This resulted

in a conclusion being made that inhibition of JP-8 degradation occurred over this interval. Referring to the modified Monod equation, as described in Section 2.5 (Equation 2-2), as the substrate concentration S is increased past the half saturation constant ($S>>K_s$), further increases in the substrate concentration will have no significant impact on microbial growth. Therefore, the conclusion of inhibition could be invalid, since the concentration of substrate (supra-CMC Alfonic and JP-8) could conceivably be maximizing the growth capacity of the microbial population present in the microcosms.

From interval 61 to 95 (375 to 600 hrs), the calculated t value was within the 0.05 level of significance interval range ($-2.365 \geq T \leq 2.365$) of the null hypothesis, therefore it was concluded that over this interval range the addition of supra-CMC Alfonic surfactant had no effect on the JP-8 biodegradation.

4.4 COMPARISON OF O_2/CO_2 RATIO

The amount of oxygen consumed and carbon dioxide evolved, by indigenous microorganisms, depends on the amount of substrate (carbon source) that is present, assuming there are adequate nutrients to support microbial metabolism. Other factors which affect the amount of carbon dioxide which evolves include: carbon which is transformed from the substrate into intermediate products and carbon converted to cell biomass. As a result, since carbon can be trapped in cell biomass and intermediate products, the amount of CO_2 that evolves does not necessarily predict the true amount of substrate that is transformed. However, the ratio of O_2 to CO_2 can provide an estimate of

how much carbon is trapped in the soil system, and how much substrate has been transformed.

Although the comparison of ratios of oxygen and carbon dioxide (O_2/CO_2) was not part of the original study, it was observed that there was a noticeable difference in the ratios seen between the different treatments of Experiment #4. Refer to Table D-1 for a summary of the mean oxygen consumption, mean carbon dioxide evolved, and ratio of O_2/CO_2 for each treatment. Table 4-1 summarizes the O_2/CO_2 ratio at the last sampling interval (95) for experiment #4.

Table 4-1 Summary of O_2/CO_2 Ratios for Exp #4 at Interval #95

Avg Blank Soil	Avg JP-8 & Soil	Avg Sub-CMC ALF & Soil	Avg CMC ALF & Soil	Avg Supra-CMC ALF & Soil	Avg JP-8 & Sub-CMC ALF & Soil	Avg JP-8 & CMC ALF & Soil	Avg JP-8 & Supra-CMC ALF & Soil
2.017	2.248	2.294	2.242	2.219	2.346	2.528	2.418

The O_2/CO_2 ratio is smallest for blank soil, and is slightly higher for JP-8, sub-CMC Alfonic, CMC Alfonic, and supra-CMC Alfonic treatments. The JP-8, sub-CMC Alfonic, CMC Alfonic, and supra-CMC Alfonic treatments all have a similar O_2/CO_2 ratio. The highest ratio (2.53) corresponds to the combined treatment of JP-8 and CMC Alfonic, which is the same treatment that was concluded to provide enhanced biodegradation of JP-8. The ratio for the CMC Alfonic combined treatment exceeds the combined treatments of sub-CMC Alfonic and JP-8, as well as supra-CMC Alfonic and JP-8 both. Figure D.1 provides a graphical representation of the O_2/CO_2 ratios for each treatment

over the entire sampling intervals. This clearly shows that the O_2/CO_2 ratio, for the JP-8 and CMC Alfonic treatments, exceeds the other treatment ratios.

It is suspected that the O_2/CO_2 ratio increases as a result of substrate (JP-8 or surfactant) addition to the soil microcosms. As a result of an increase in substrate concentration, the apparent growth of microorganisms is stimulated. The stimulated growth occurs as a result of microorganisms consuming oxygen to oxidize the added substrates. Some of the carbon present in the original substrate components is converted to CO_2, while some of the carbon is trapped in the soil system as biomass and other substituents of the original substrate components. It is postulated that an increase in the ratio indicates that more of the substrate has been transformed (biodegraded) into substituent compounds and cell biomass, and is not being evolved as CO_2. Therefore it is concluded, based on this postulate, that the increased O_2/CO_2 ratio, for the combined treatment of JP-8 and CMC Alfonic, provides further proof that JP-8 biodegradation has been enhanced, and corresponds to more of the JP-8 compounds being converted to substituent compounds and additional biomass.

5. Conclusions and Recommendations

5.1 Conclusions

Biodegradation of JP-8 fuel had been previously studied by Baker (1995) and Totten (1995), and it was determined that the JP-8 was readily degradable in three different soil types. However biodegradation can require years for complete site cleanup. The primary reason that biodegradation requires such long periods of time is that it depends on not only the chemical properties of the contaminant, but the physical characteristics of the site as well. The purpose of this research was to determine if it was possible to enhance the biodegradation of simulated JP-8 fuel contaminated soil, by adding surfactants. A Micro-Oxymax respirometer was used to measure the amount of oxygen uptake for each of the microcosm treatments investigated. From the oxygen uptake curves, enhanced or inhibited biodegradation was determined. The purpose of this study was to determine if the oxygen uptake, and ultimately, biodegradability of HOCs would be increased through surfactant addition.

Respirometer repeatability was proven based on the fact that the mean oxygen uptake curves for replicates, from the same experimental runs, overlie one another. While reproducibility was not as easy to conclude, the respirometer was determined to be fairly reproducible based on the fact that the uncontaminated and the JP-8 soil treatments had small standard errors over the entire sampling interval. The respirometer appeared not to be reproducible for treatments which added CMC and supra-CMC Alfonic 810-4.5.

However, since measurement error was introduced from using separate surfactant stock solutions for each experimental run, this error was the cause of non-reproducibility rather than the respirometer itself. Therefore, the respirometer was determined to be repeatable within identical experiments, and reproducible between separate experiments, assuming that measurement error associated with microcosm additives was kept to a minimum.

Preliminary respirometer studies were conducted to ensure that JP-8 contaminated soil does biodegrade, and to validate the findings of Baker (1995) and Totten (1995). A statistical hypothesis test, using the t distribution, was conducted to determine if biodegradation occurred. The null hypothesis was that no degradation occurred, and the difference in the mean cumulative oxygen uptake of JP-8 contaminated and uncontaminated soil was 0. At all sampling intervals, the difference between the mean cumulative oxygen uptake for JP-8 contaminated and uncontaminated soil microcosms, had a calculated T value which exceeded t_{crit}, and resulted in biodegradation being concluded. Therefore, JP-8 contaminated microcosms were found to be degraded by the indigenous soil microbes, present in the Kittyhawk silt soil used.

Three levels (sub-CMC, CMC, and supra-CMC) of surfactant were used to determine if there was no effect, an enhancement, or inhibition of microbial metabolism, refer to Table 5-1 for a summary of the results. Surfactant enhanced biodegradation of JP-8 was investigated by also performing a statistical hypothesis test about the difference of the means.

Table 5-1 Summary of Results for Surfactant Effects on JP-8 Contaminated Soil.

Surfactant Treatment Level	Observed Effect at Sampling Intervals		
	No Effect	Inhibition	Enhancement
Sub-CMC Alfonic	1 - 95	-	-
CMC Alfonic	1 - 82	-	83 - 95
Supra-CMC Alfonic	61 - 95	1 - 60	-

Note: Sampling Interval = 6.3 hrs

The null hypothesis was that no effect on JP-8 biodegradation would be seen, and the difference of the mean oxygen uptakes for the combined treatments of JP-8 and surfactant and the individual treatments would be 0. Sub-CMC Alfonic addition resulted in no effect on the biodegradation of JP-8 over all sampling intervals. Whereas, CMC Alfonic addition was concluded to enhance the biodegradation of JP-8 from sampling interval 83 to 95. The supra-CMC Alfonic addition resulted in inhibition from sampling interval 1 to 60, and no effect over the remaining sampling intervals. Therefore CMC Alfonic addition was the only concentration which was concluded to enhance the biodegradation of JP-8.

5.2 Problems Using Respirometry Alone

Respirometry does not provide the analytical tools necessary for determining which carbon substrates are being metabolized by microorganisms. Since the oxygen uptake curves are not more definitive, between individual and combined treatments, for determining enhancement, a separate analytical technique will be required to further prove what source

of carbon was being used by the indigenous microorganisms. Gas Chromatography (GC) is one method which can be employed to assist in analysis of the different microcosm treatments. GC can provide for definitive identification of what carbon species are present in the soil samples. GC analysis can be accomplished before and after the microbial studies with the respirometer. This will allow for comparison of chromatograms before and after the experiment, and will identify which carbon species were present at the beginning and end of the degradation period. A chromatogram provides a distribution of carbon species which are present in a sample being analyzed. This data can be used to determine what carbon sources are being utilized by the microorganisms, and show whether the surfactants or the JP-8 fuel components are being transformed.

5.3 Follow-On Research

There are four recommendations for follow-on research: using a weathered contaminated soil sample, using GC analytical methods, using a one or two component contaminant rather than a contaminant with numerous compounds (like JP-8), and generating sorption isotherms for the respective surfactants and soils used.

5.3.1 Weathered Soil Sample

One recommendation for future studies is that a weathered contaminated soil be obtained to conduct respirometry analysis. This would allow a better understanding of an actual contaminated site, and would provide a sample that would have limited volatile components left in the soil. The weathered soil would have already undergone some biodegradation already, and the more recalcitrant and strongly sorbed components would

be still trapped in the soil. As a result, the biodegradation of weathered soil, and determination of surfactant enhancement would provide a better conclusion about using surfactants to enhance biodegradation for an actual site.

5.3.2 Gas Chromatography

As discussed previously, gas chromatography will provide a better understanding of exactly which components of the contaminant are being utilized by the indigenous microorganisms. Also, this will provide for a complete mass balance of the carbon in the soil microcosms.

5.3.3 Single or Two Component Contaminant With Low Volatility

By using a single component contaminant, with a low volatility, the analysis of data will be greatly simplified. By using one or two compounds, rather than JP-8 fuel, which has numerous compounds, allows for a much simpler mass balance to be conducted before and after the biodegradation experiments. This will also make the chemical analysis much easier also.

5.3.4 Sorption Isotherms for Surfactants Used

By using a High Performance Liquid Chromatograph, a sorption isotherm can be established for the surfactants and soil used for the biodegradation studies. This will give a better understanding of the amount of surfactant which sorbs to the soil of interest, and

will allow for determining a more accurate amount of surfactant that must be added to achieve the desired concentration in the aqueous phase.

Appendix A RESPIROMETER OXYGEN UPTAKE CURVES FOR EACH TREATMENT

The following pages contain the cumulative oxygen consumption curves for all of the treatments conducted in the experiments. The oxygen curves are shown, since they were used to measure the amount of biodegradation that occurred. The carbon dioxide curves are identical to the oxygen curves, except at a smaller scale for the amount of cumulative carbon dioxide evolved. A graph of each treatments mean along with its respective standard error are also shown. These curves will be used to evaluate the reproducibility of the respirometer.

LIST OF FIGURES

Figure A.1 Uncontaminated Kittyhawk Silt Soil Microcosm, Cumulative Oxygen Uptake.

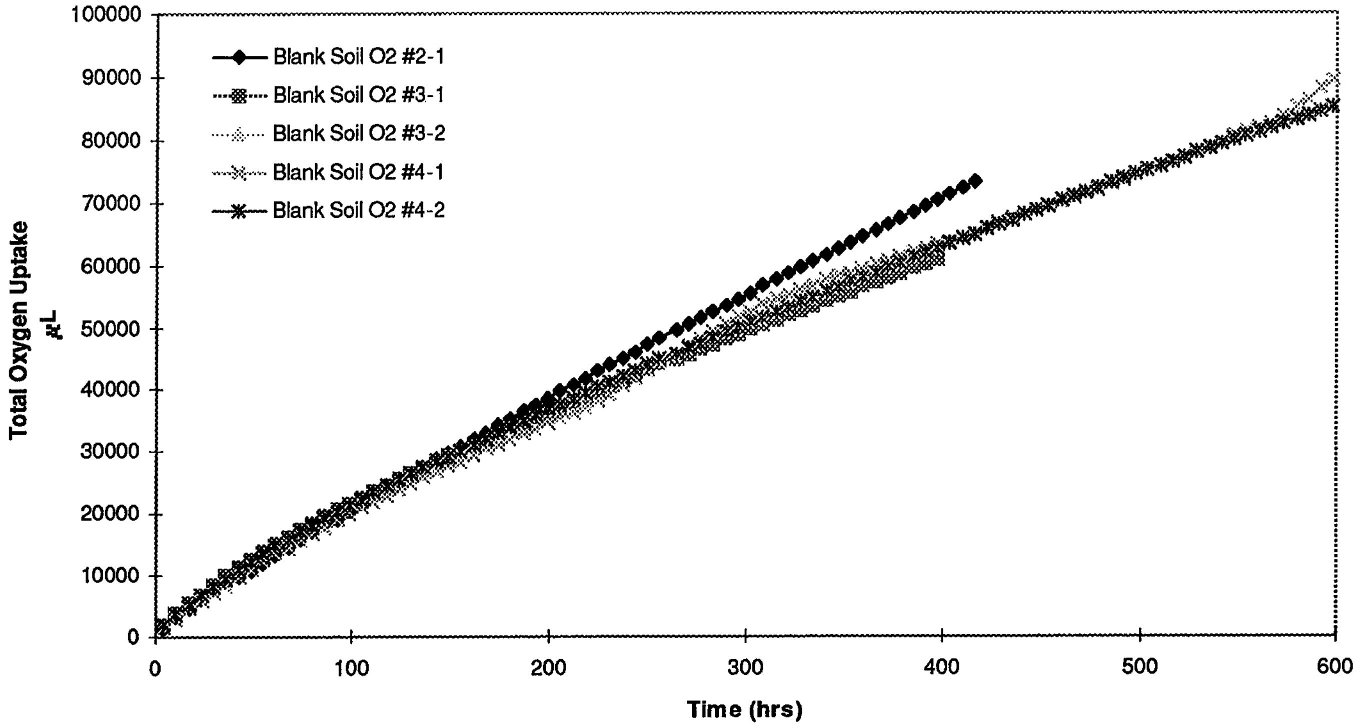


Figure A.2 Uncontaminated Kittyhawk Silt Soil Microcosm, Mean Cumulative Oxygen Uptake and Standard Error.

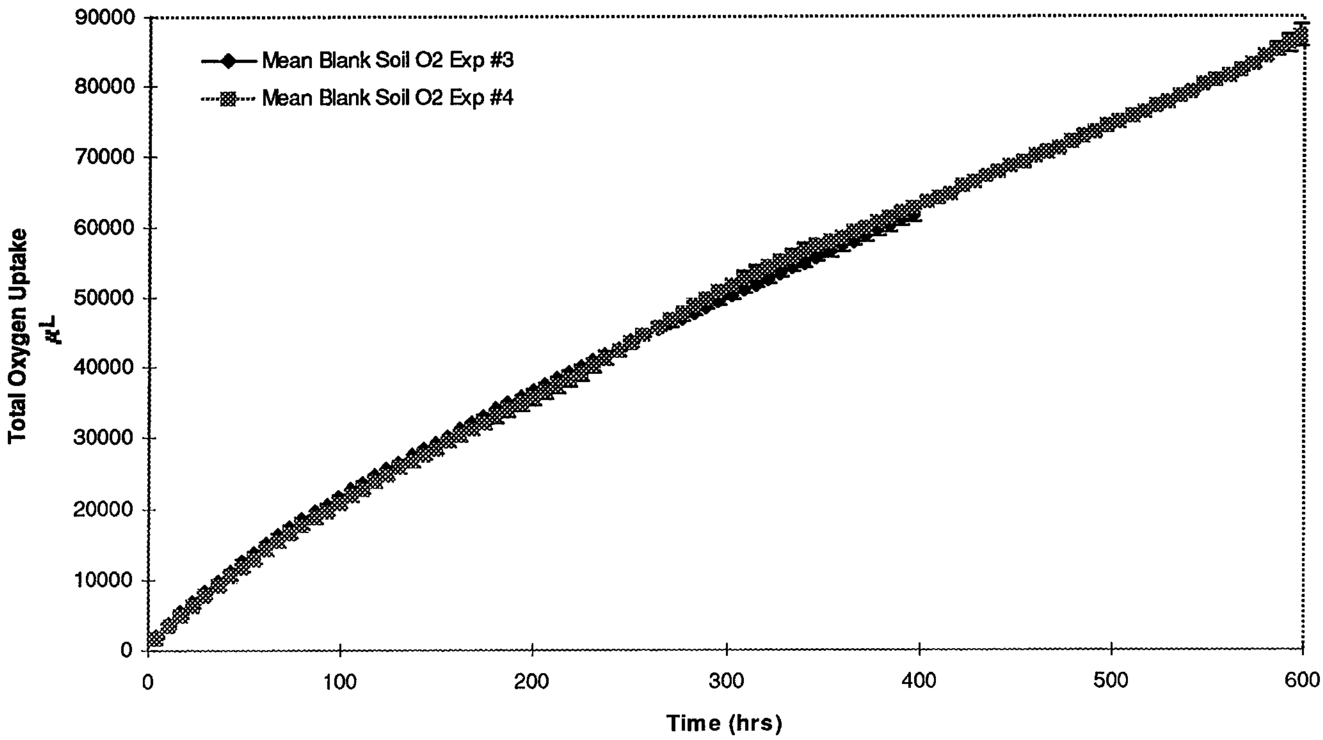


Figure A.3 Sub-CMC Alfonic 810-4.5 Addition to Soil Microcosms, Cumulative Oxygen Uptake.

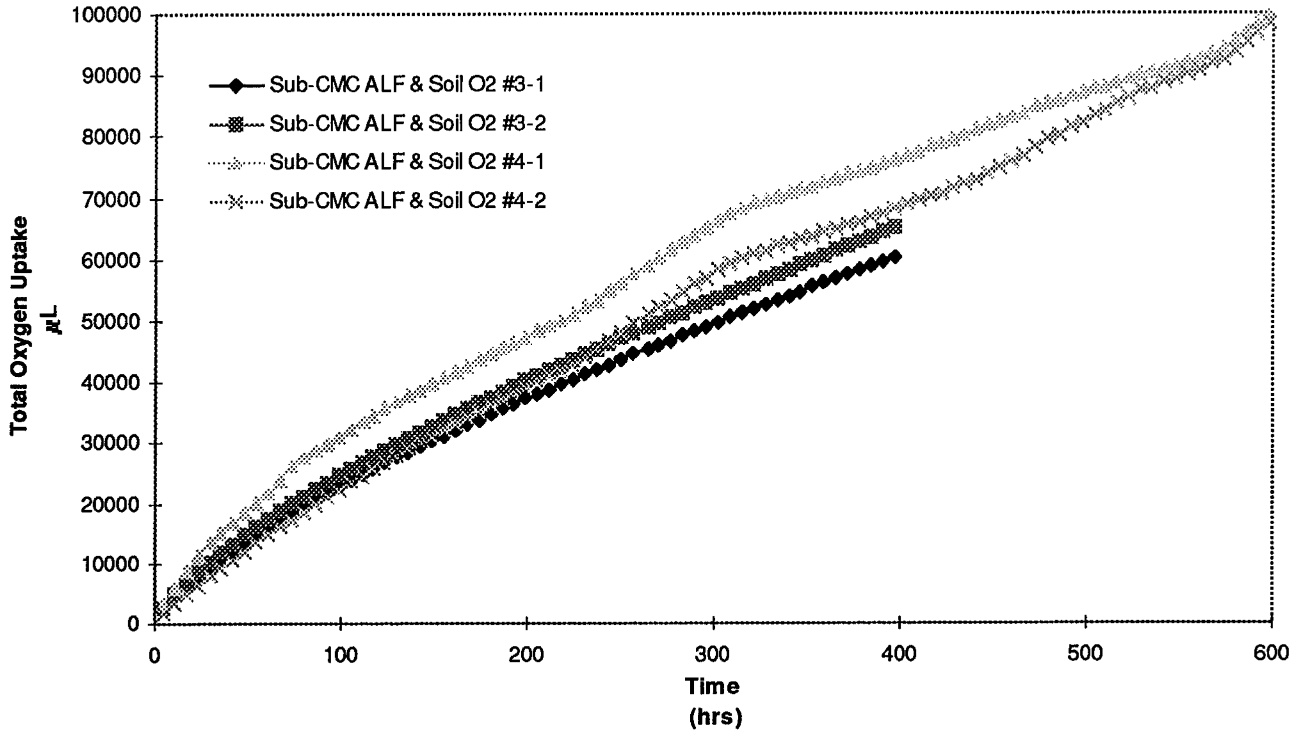


Figure A.4 Sub-CMC Alfonic 810-4.5 Addition to Soil Microcosms, Mean Cumulative Oxygen Uptake and Standard Error.

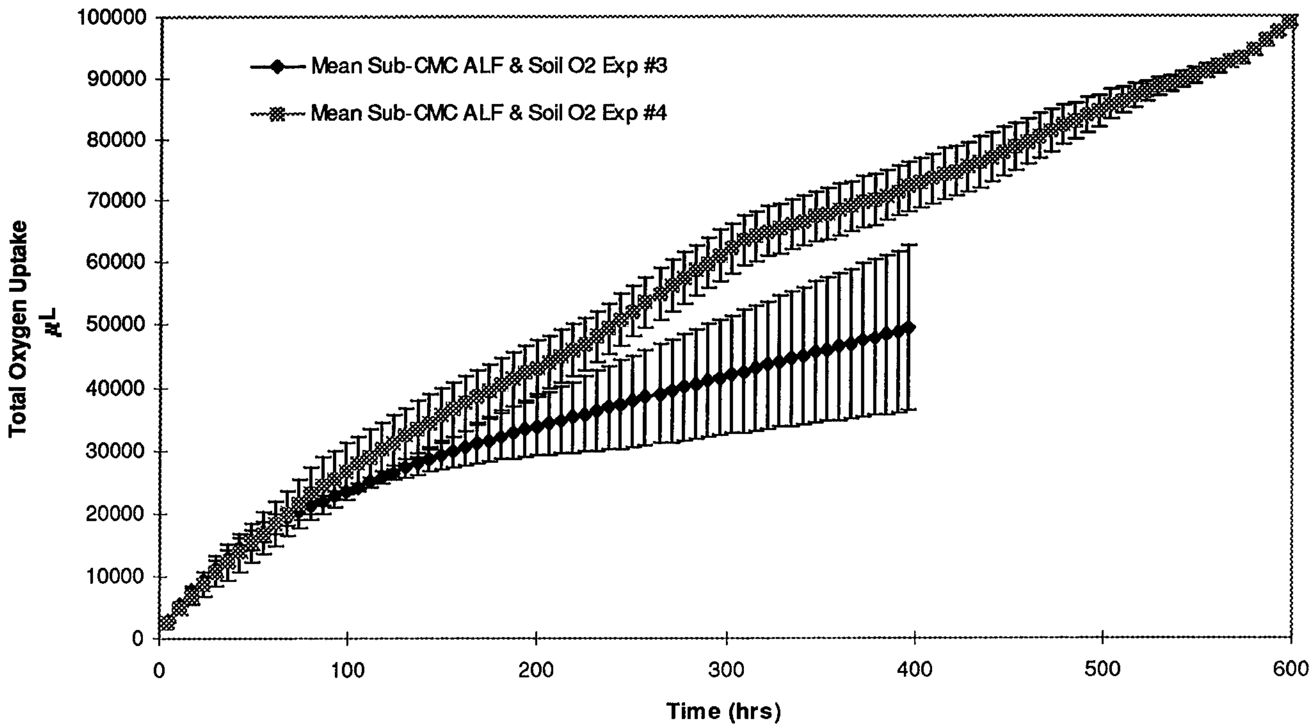


Figure A.5 CMC Alfonic 810-4.5 Addition to Soil Microcosms, Cumulative Oxygen Uptake.

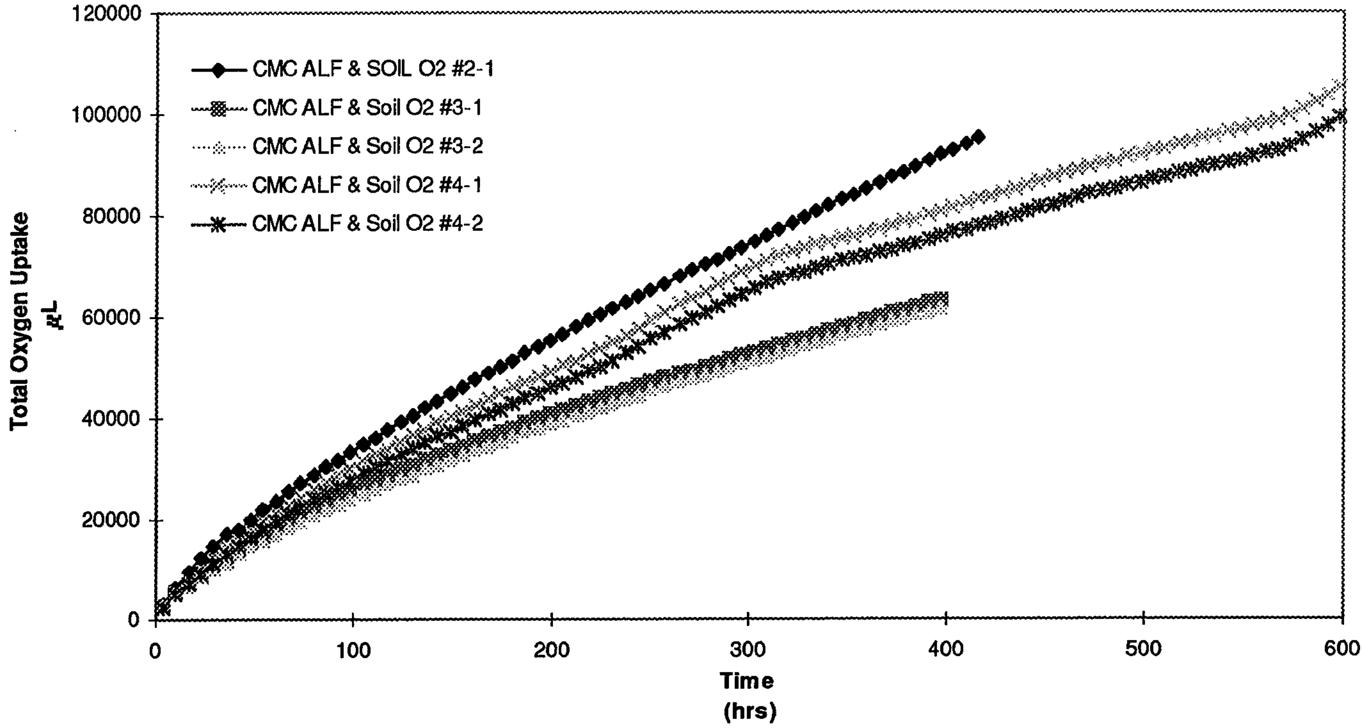


Figure A.6 CMC Alfonic 810-4.5 Addition to Soil Microcosms, Mean Cumulative Oxygen Uptake and Standard Error.

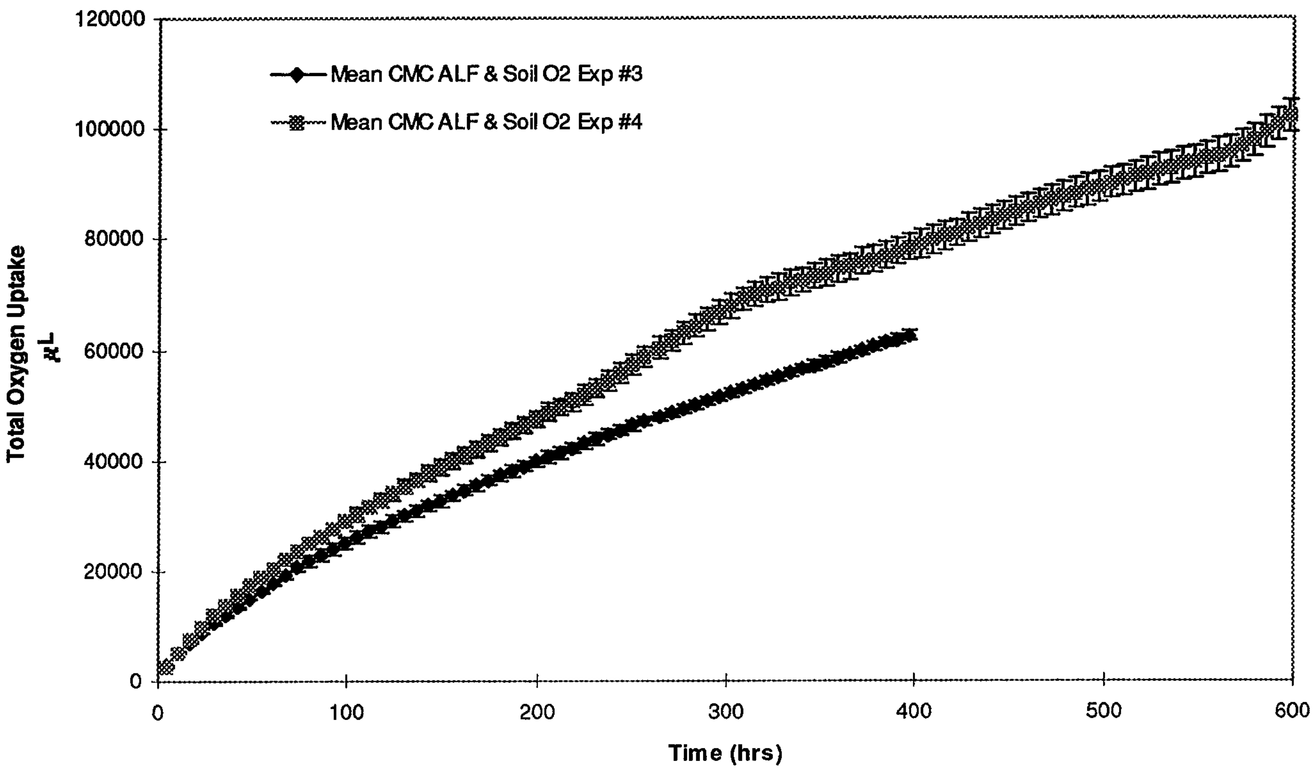


Figure A.7 Supra-CMC Alfonic 810-4.5 Addition to Soil Microcosms, Cumulative Oxygen Uptake.

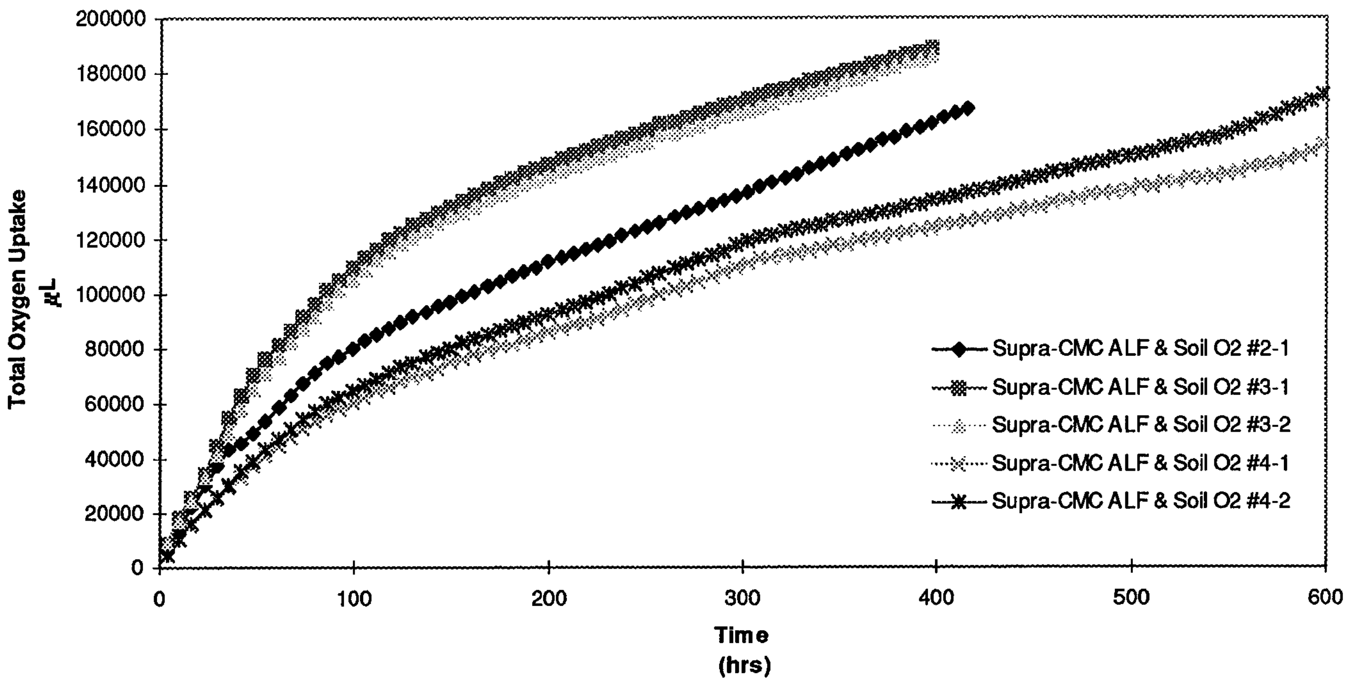


Figure A.8 Supra-CMC Alfonic 810-4.5 Addition to Soil Microcosms, Mean Cumulative Oxygen Uptake and Standard Error.

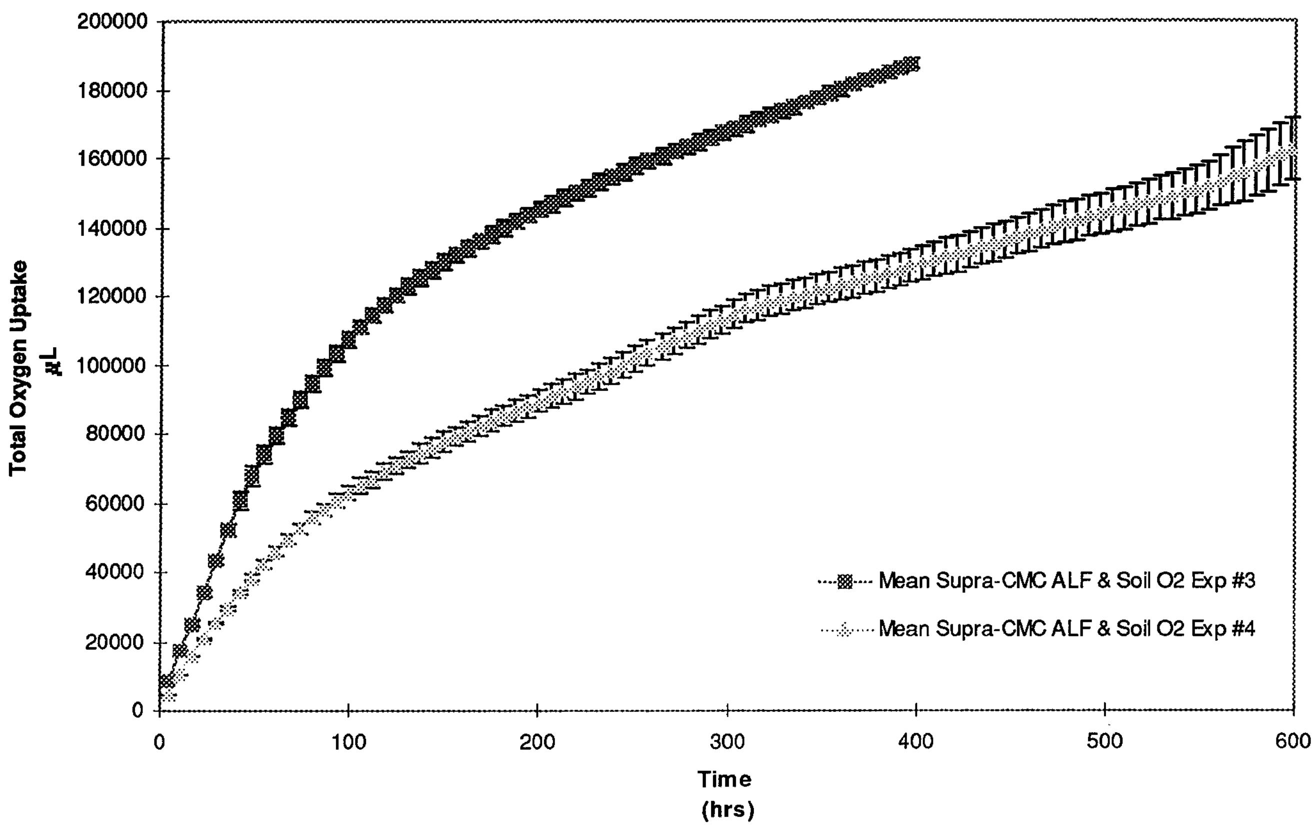


Figure A.9 Biodegradation of JP-8 Contaminated Soil Microcosms, Cumulative Oxygen Uptake.

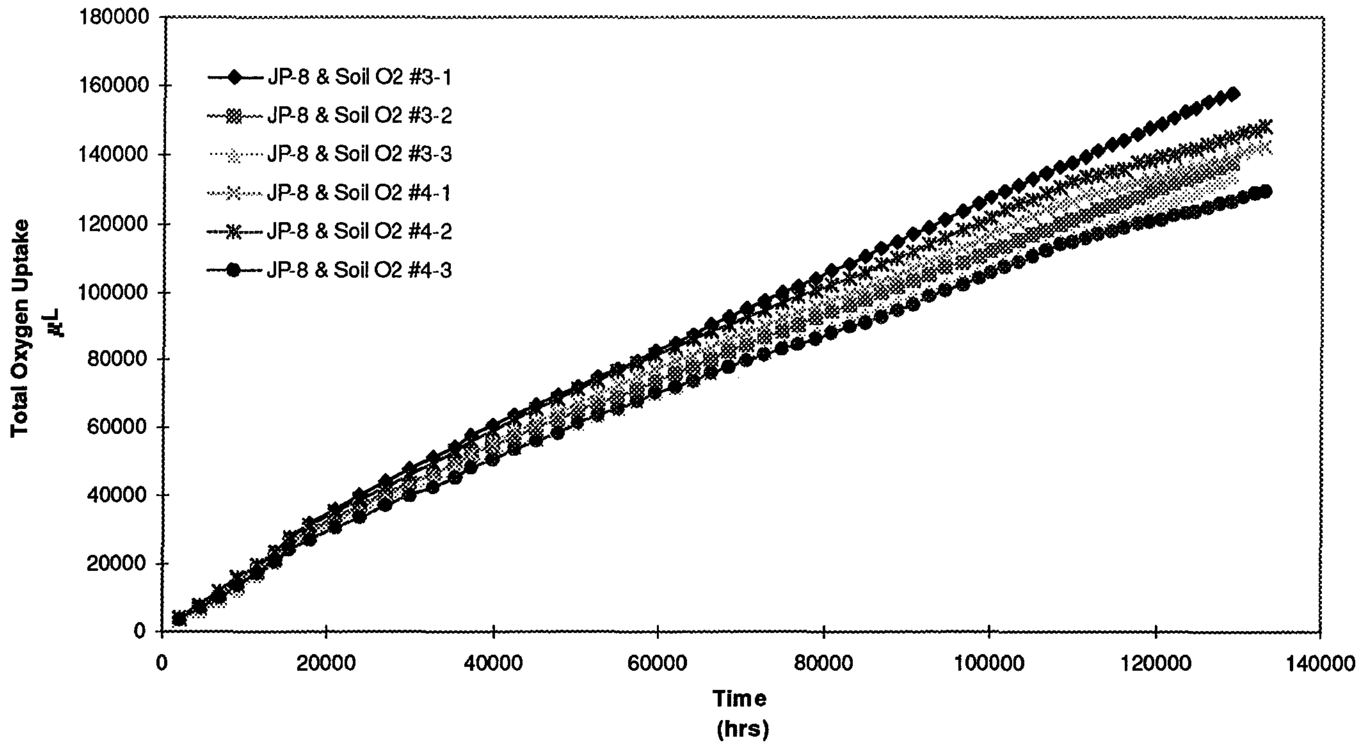


Figure A.10 Biodegradation of JP-8 Contaminated Soil Microcosms, Mean Cumulative Oxygen Uptake and Standard Error.

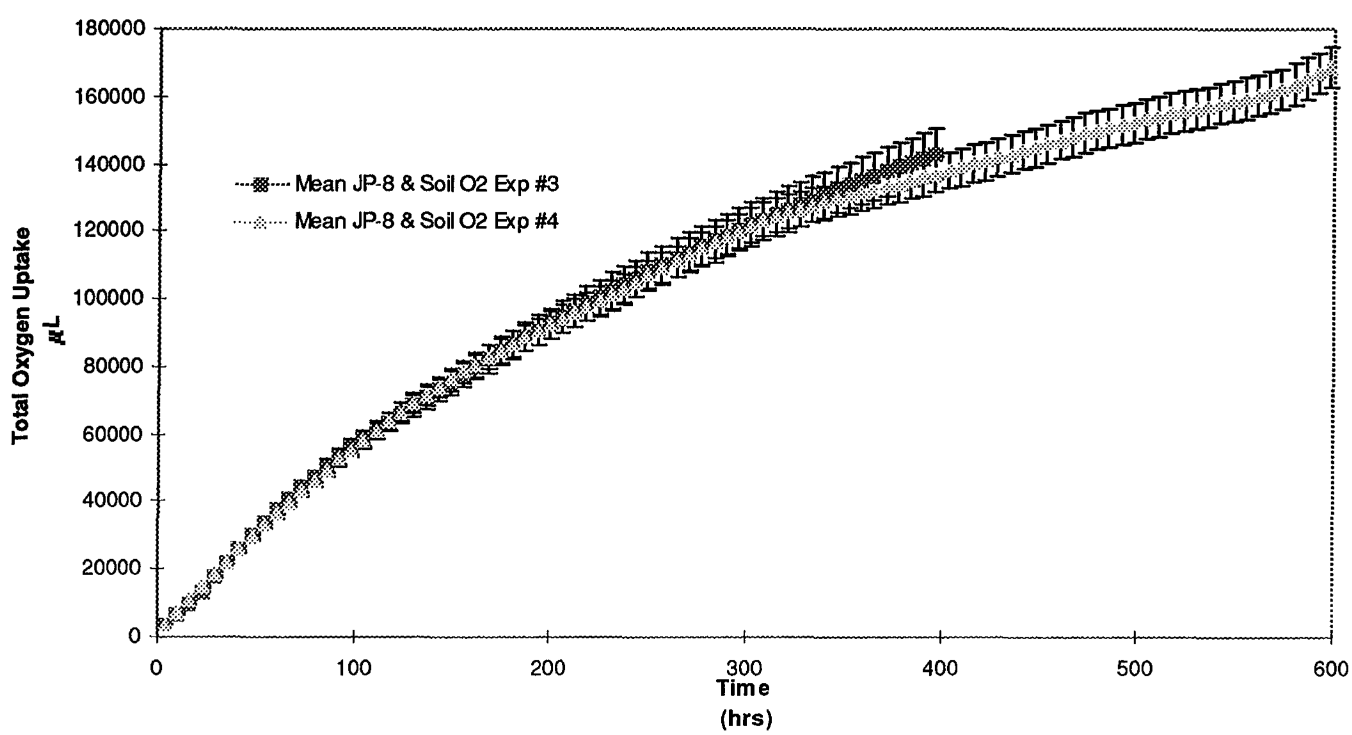


Figure A.11 Biodegradation of JP-8 Contaminated Soil Microcosms with Sub-CMC Alfonic 810-4.5 Addition for Enhancement, Cumulative Oxygen Uptake.

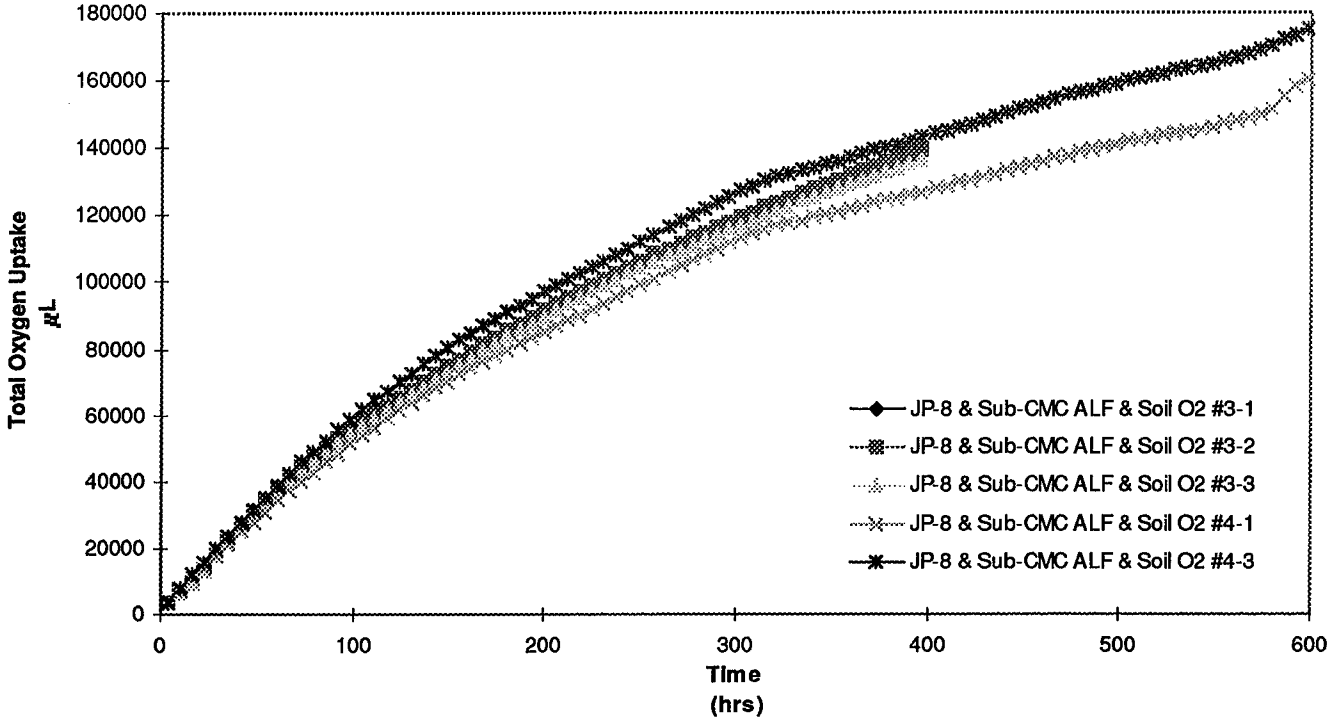


Figure A.12 Biodegradation of JP-8 Contaminated Soil Microcosms with Sub-CMC Alfonic 810-4.5 Addition for Enhancement, Mean Cumulative Oxygen Uptake and Standard Error.

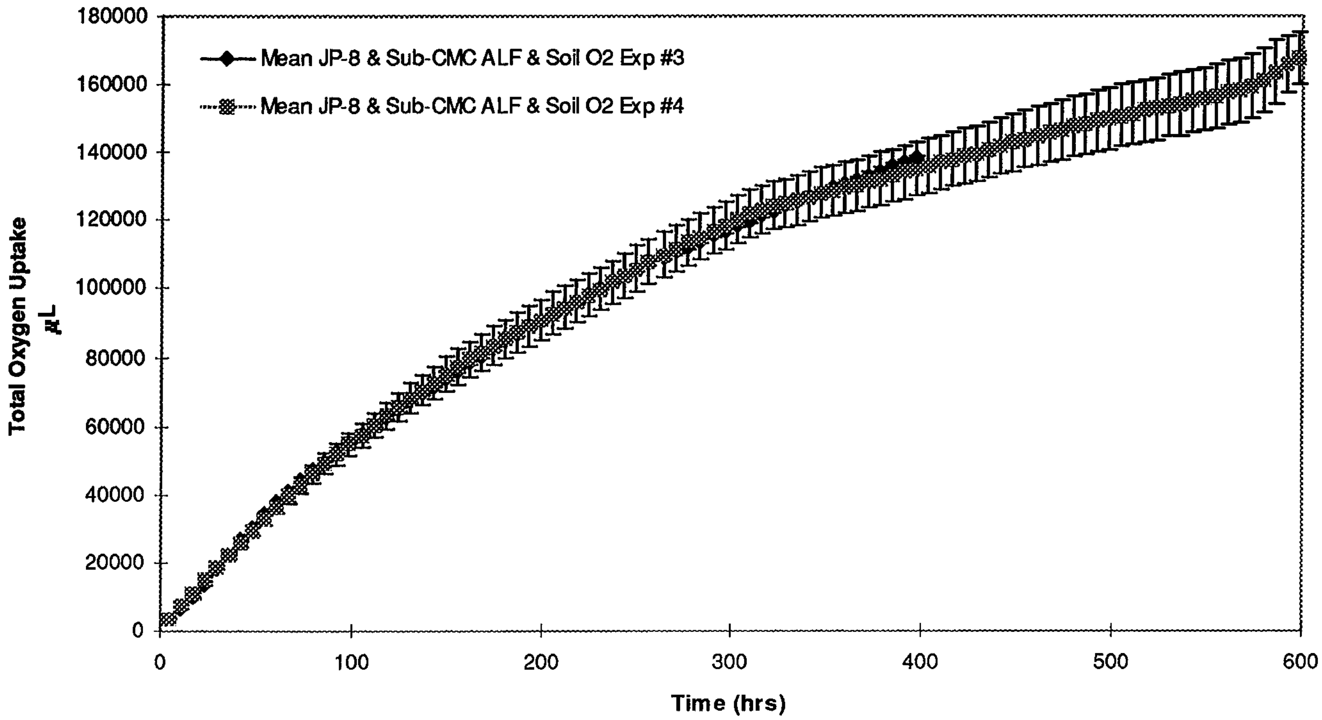


Figure A.13 Biodegradation of JP-8 Contaminated Soil Microcosms with CMC Alfonic 810-4.5 Addition for Enhancement, Cumulative Oxygen Uptake.

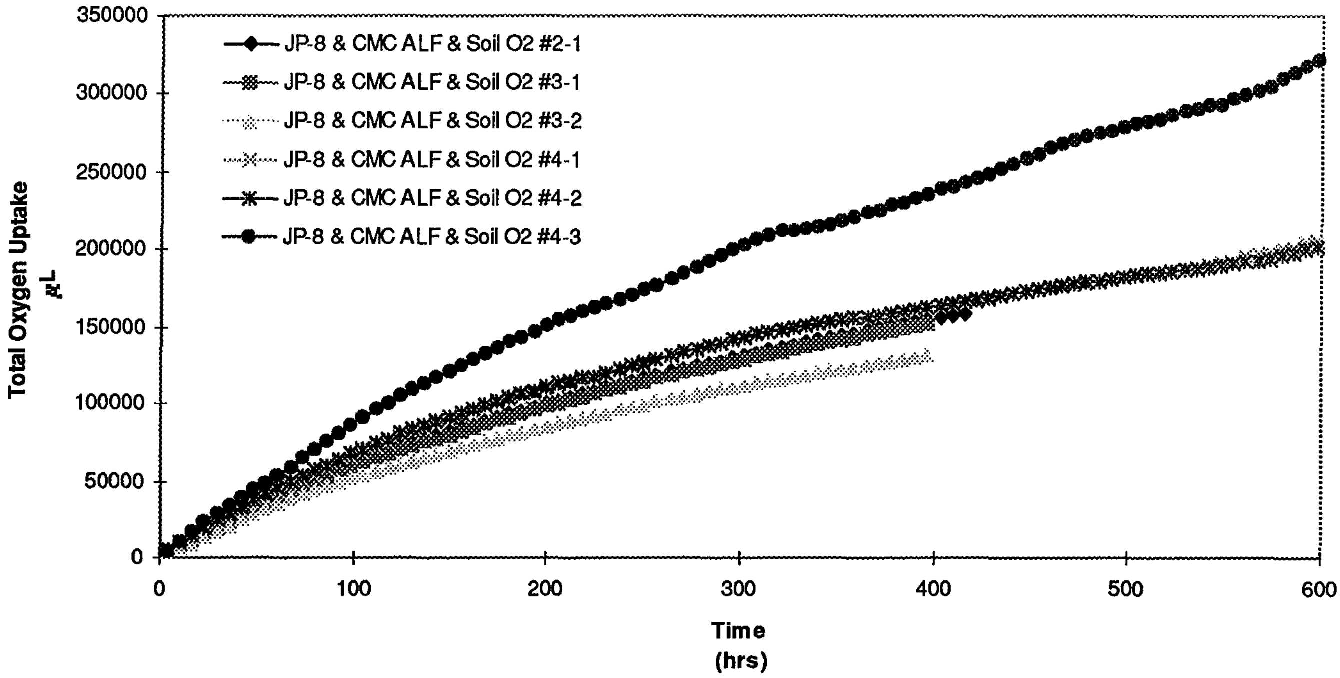


Figure A.14 Biodegradation of JP-8 Contaminated Soil Microcosms with CMC Alfonic 810-4.5 Addition for Enhancement, Mean Cumulative Oxygen Uptake and Standard Error.

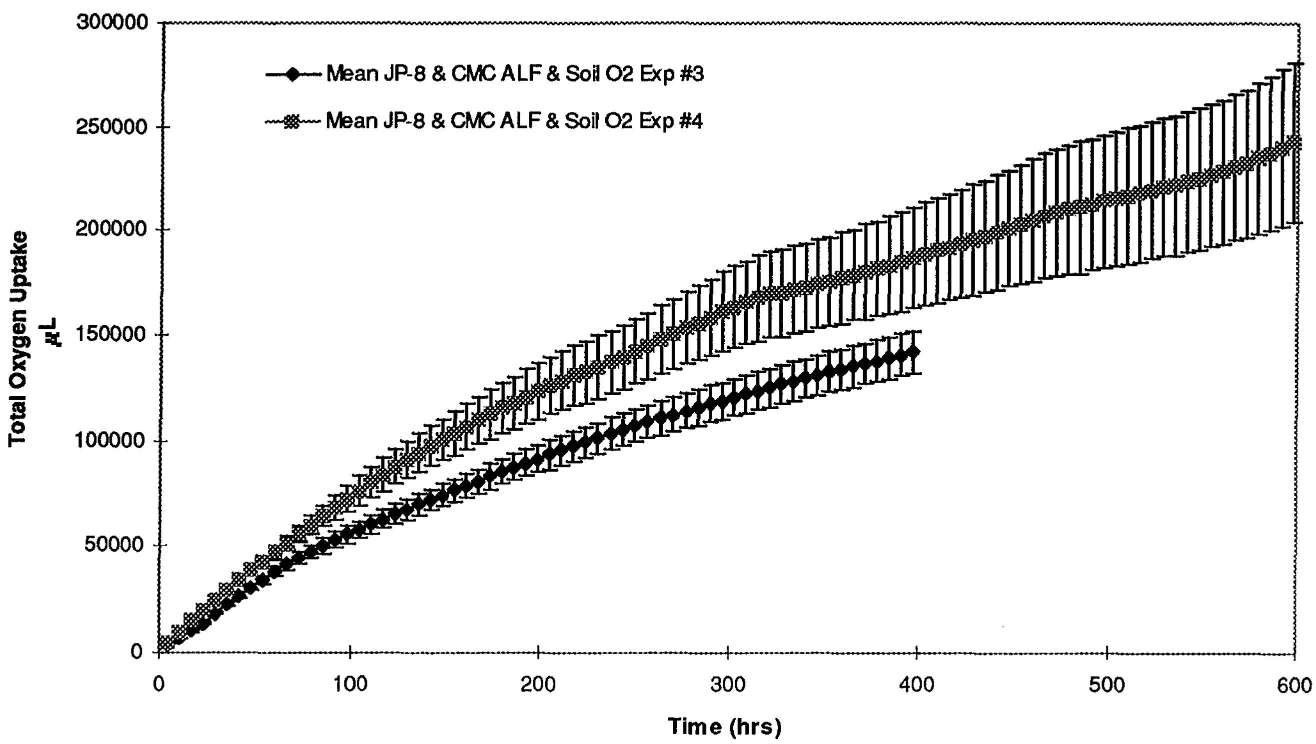


Figure A.15 Biodegradation of JP-8 Contaminated Soil Microcosms with Supra-CMC Alfonic 810-4.5 Addition for Enhancement, Cumulative Oxygen Uptake.

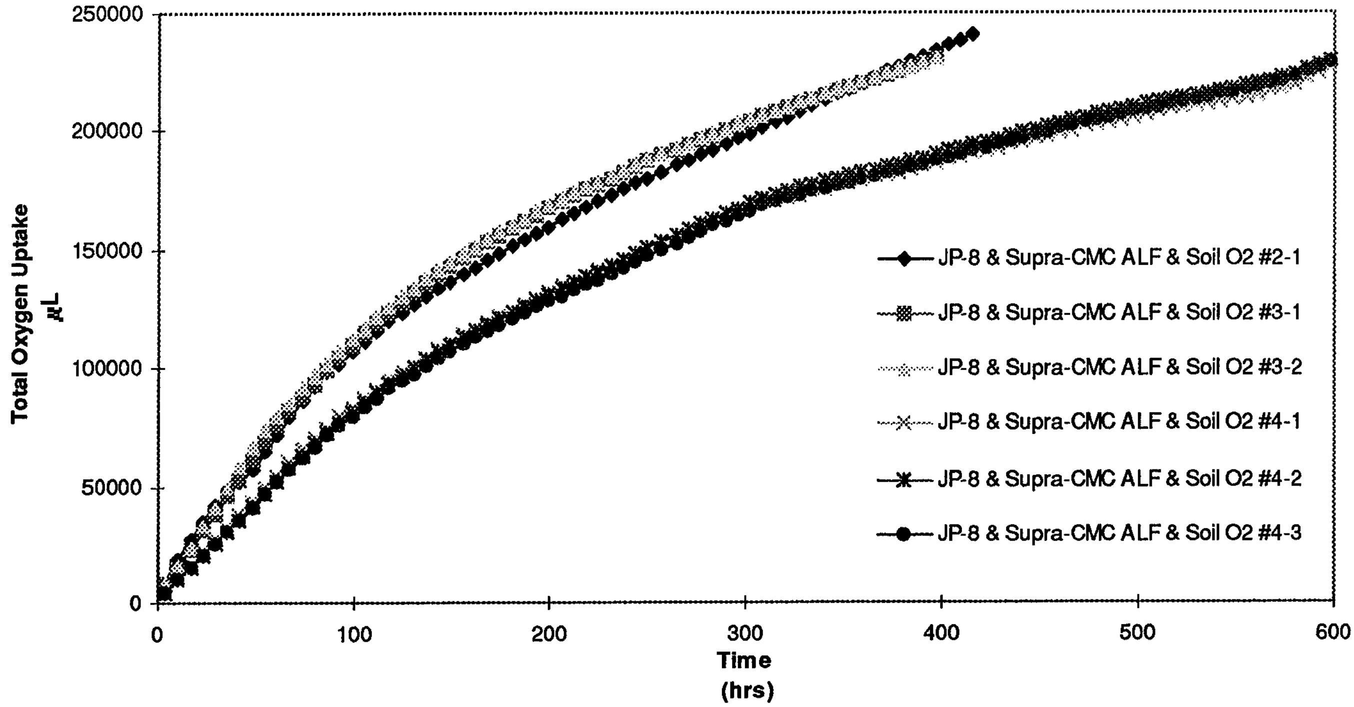
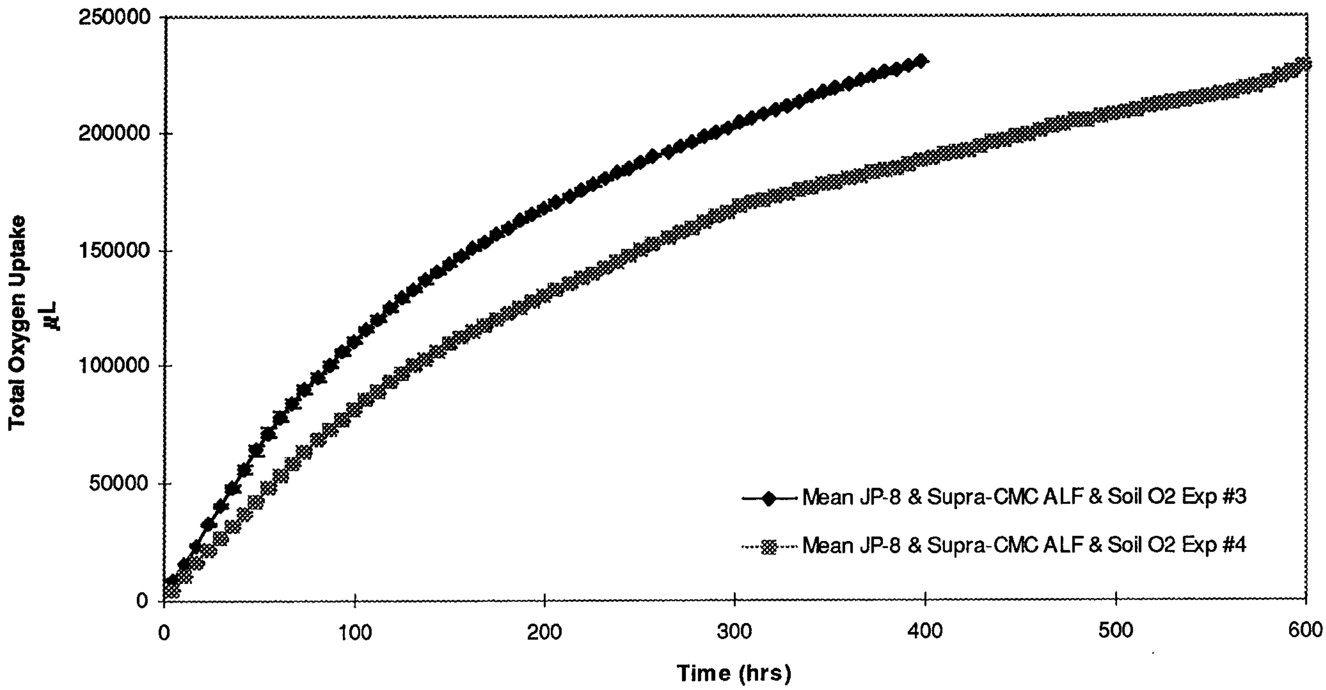


Figure A.16 Biodegradation of JP-8 Contaminated Soil Microcosms with Supra-CMC Alfonic 810-4.5 Addition for Enhancement, Mean Cumulative Oxygen Uptake and Standard Error.



Appendix B STATISTICAL OXYGEN UPTAKE DATA TABLES FOR DETERMINING ENHANCEMENT OF JP-8 BIODEGRADATION

The following pages contain tables summarizing the statistical parameters necessary to determine whether enhancement of JP-8 contaminated soil occurred as a result of surfactant addition. Each table individually summaries the effect for addition of sub-CMC, CMC, and supra-CMC Alfonic 810-4.5 ethoxylate nonionic surfactant. The mean oxygen uptake curves along with their respective standard deviation and variance are listed over the 95 intervals which were sampled. The difference of the means and the standard deviation used to calculate the t value are also given. The t value is ultimately compared to the critical t value, by using a logical if then statement in Excel, to determine if there was no effect, inhibition, or enhancement. The results are listed in the last column over the entire interval.

List of Tables

TABLE B-1 DATA FOR DETERMINING SUB-CMC ALFONIC ENHANCEMENT OF JP-8 BIODEGRADATION

Int	Mean Blank Soil O2	Std Dev of Blank Soil O2	Var Blank Soil O2	Mean Sub CMC ALF & Soil O2	Std Dev Sub-CMC ALF & Soil O2	Var Sub-CMC ALF & Soil O2	Mean JP-8 & Soil O2	Std Dev JP-8 & Soil O2	Var JP-8 & Soil O2	Mean JP-8 & Sub-CMC ALF & Soil O2	Std Dev JP-8 & Sub-CMC ALF & Soil O2	Var JP-8 & Sub-CMC ALF & Soil O2	$\bar{X}_{JP8/SURF} - \bar{X}_{JP8} - \bar{X}_{SURF} + \bar{X}_{SOIL}$	Std Dev of Test Statistic	Calc T Val (|T Crit| = 2.447)	Effect of Surfactant Addition
1	1686.5	225.6	50880.5	2454.5	1086.823	1181185	3607.7	330.443	109192.3	3751.5	219.9102	48360.5	-783.66667	822.563	-0.953	NO EFFECT
2	3291.5	371.2	137812.5	4673	1920.502	3688328	7262	529.289	280147	7550	530.3301	281250	-1356	1465.29	-0.925	NO EFFECT
3	4814	468.1	219122	6763	2699.734	7288562	10918	749.36	561541	11279.5	857.7205	735684.5	-1918.5	2075.78	-0.924	NO EFFECT
4	6263.5	552.3	304980.5	8800	3471.894	12054050	14650	991.877	983820.3	15019	1186.525	1407842	-2557.6667	2685.4	-0.952	NO EFFECT
5	7678	627.9	394272	10691	4067.278	16542752	18395	1246.68	1554220	18740.5	1525.229	2326325	-3111.1667	3185.87	-0.977	NO EFFECT
6	9041	694.4	482162	12309.5	4322.544	18684385	22112	1516.81	2300716	22395.5	1844.842	3403441	-3475.6667	3471.58	-1.001	NO EFFECT
7	10364	758.0	574592	13774	4381.234	19195208	25821	1792.44	3212850	26019	2162.333	4675682	-3748.3333	3646.05	-1.028	NO EFFECT
8	11649	823.8	678612.5	15375	4702.26	22111250	29458	2066.77	4271557	29559.5	2482.652	6163561	-4207.8333	3987.55	-1.055	NO EFFECT
9	12886	869.7	756450	16851.5	4865.602	23674081	33019	2340.13	5476188	33028	2810.042	7896338	-4571.5	4241.32	-1.078	NO EFFECT
10	14080	917.8	842402	18329.5	5043.793	25439845	36468	2614.05	6833257	36375	3123.998	9759362	-4991.8333	4505.39	-1.108	NO EFFECT
11	15227	946.1	895122	19924.5	5452.5	29729761	39811	2884.1	8318006	39630	3432.296	11780658	-5547.8333	4896.47	-1.133	NO EFFECT
12	16437	900.1	810264.5	21673.5	6120.009	37454513	43049	3112.2	9685790	42924.5	3899.694	15207613	-5998.3333	5474.01	-1.096	NO EFFECT
13	17554	937.6	879138	23150	6388.003	40806578	46255	3377.87	11409994	46034	4193.143	17582450	-6479.6667	5782.51	-1.121	NO EFFECT
14	18645	980.8	961884.5	24332.5	6308.1	39792121	49352	3638.08	13235611	49035.5	4485.178	20116825	-6698	5903.16	-1.135	NO EFFECT
15	19708	996.3	992640.5	25511.5	6233.146	38852113	52403	3893.07	15155977	51992	4772.971	22781250	-6919.5	6030.34	-1.147	NO EFFECT
16	20756	1018.2	1036800	26694	6242.339	38966792	55361	4135.44	17101842	54865	5058.642	25589858	-7154.3333	6204.62	-1.153	NO EFFECT
17	21769	1014.0	1028178	27867	6238.096	38913842	58258	4372.41	19117933	57675	5334.414	28455968	-7398	6369.59	-1.161	NO EFFECT
18	22786	1032.4	1065800	29025	6219.711	38684808	61075	4599.25	21153121	60414	5610.185	31474178	-7630.3333	6531.73	-1.168	NO EFFECT
19	23755	1005.5	1011042	30148	6202.741	38473992	63813	4819.94	23231780	63068.5	5872.522	34486513	-7848.1667	6688.03	-1.173	NO EFFECT
20	24736	1020.4	1041125	31254	6202.741	38473992	66476	5032.9	25330066	65659.5	6131.323	37593121	-8056.8333	6855.47	-1.175	NO EFFECT
21	25695	1033.1	1067261	32337.5	6182.235	38220025	69039	5236.19	27417703	68151	6380.932	40716288	-8261.5	7010.07	-1.179	NO EFFECT
22	26638	1047.2	1096681	33420	6150.415	37827602	71529	5435.43	29543908	70581.5	6622.055	43851613	-8470.8333	7157.93	-1.183	NO EFFECT
23	27575	1067.7	1140050	34505.5	6111.524	37350725	73949	5629.28	31688760	72941.5	6844.087	46841521	-8692.6667	7295.83	-1.191	NO EFFECT
24	28509	1087.5	1182722	35546.5	6090.311	37091885	76287	5816.85	33835711	75228	7062.583	49880072	-8865.5	7440.16	-1.192	NO EFFECT
25	29424	1140.6	1300885	36543	6071.219	36859698	78490	5992.59	35911092	77392.5	7262.694	52746721	-9023.8333	7577.86	-1.191	NO EFFECT
26	30339	1205.6	1453513	37531.5	6043.642	36525605	80624	6156.17	37898413	79496.5	7459.269	55640701	-9173	7709.91	-1.190	NO EFFECT
27	31263	1272.1	1618201	38518	6017.479	36210050	82713	6325.18	40007844	81562	7646.653	58471298	-9306	7841.28	-1.187	NO EFFECT
28	32176	1344.9	1808802	39469.5	6002.629	36031561	84711	6486.95	42080577	83542	7814.944	61073352	-9413.1667	7967.67	-1.181	NO EFFECT
29	33080	1407.1	1980050	40418.5	5992.73	35912813	86689	6643.1	44130820	85506	7983.236	63732050	-9516.1667	8094.61	-1.176	NO EFFECT
30	33976	1460.2	2132113	41363	5973.638	35684352	88604	6792.17	46133587	87404.5	8142.335	66297613	-9619.5	8211.88	-1.171	NO EFFECT
31	34865	1491.3	2223941	42296.5	5957.375	35490313	90461	6932.46	48059037	89252	8291.534	68749538	-9695.5	8321.75	-1.165	NO EFFECT
32	35738	1496.9	2240845	43232.5	5929.09	35154113	92321	7067.86	49954610	91097	8428.713	71043200	-9777.1667	8418.47	-1.161	NO EFFECT
33	36615	1519.6	2309101	44176	5907.17	34894658	94132	7196.93	51795820	92896	8560.235	73277618	-9871.6667	8515.04	-1.159	NO EFFECT
34	37470	1524.5	2324168	45090	5881.714	34594562	95893	7327.66	53694580	94651.5	8682.564	75386921	-9939.8333	8605.29	-1.155	NO EFFECT
35	38337	1533.7	2352281	46024	5853.43	34262642	97644	7447.35	55463004	96382	8794.994	77351922	-10034	8687.41	-1.155	NO EFFECT
36	39236	1519.6	2309101	46983	5823.731	33915848	99307	7562.09	57185144	98032.5	8884.797	78939613	-10096.833	8754.66	-1.153	NO EFFECT
37	40224	1400.1	1960200	48102	5815.246	33817088	101101	7667.79	58795075	99838.5	8992.277	80861045	-10130.5	8827.09	-1.148	NO EFFECT
38	41276	1193.6	1424672	49360.5	5810.296	33759545	102979	7763.42	60270633	101731.5	9092.686	82676941	-10175.667	8889.36	-1.145	NO EFFECT
39	42368	893.1	797584.5	50731	5798.276	33620000	104947	7854.24	61689112	103718.5	9188.853	84435013	-10223.167	8943.68	-1.143	NO EFFECT
40	43542	623.0	388080.5	52179.5	5786.255	33480745	107002	7948.3	63175500	105786.5	9287.848	86264113	-10294.333	9006.94	-1.143	NO EFFECT
41	44698	371.9	138338	53592.5	5782.012	33431665	109018	8041.47	64665163	107796.5	9385.428	88086265	-10379	9076.52	-1.144	NO EFFECT
42	45757	19.8	392	54987.5	5780.598	33415313	111024	8131.99	66129194	109784.5	9473.11	89739805	-10484.333	9144.44	-1.147	NO EFFECT
43	46783	270.1	72962	56309.5	5776.355	33366281	112940	8224.05	67634990	111677.5	9562.205	91435765	-10597.667	9218.6	-1.150	NO EFFECT
44	47772	512.7	262812.5	57556.5	5770.698	33300961	114741	8305.95	68988761	113466.5	9649.886	93120305	-10697.333	9291.84	-1.151	NO EFFECT
45	48766	702.2	493024.5	58771	5772.82	33325448	116480	8386.38	70331436	115188	9738.275	94833992	-10801.333	9368.57	-1.153	NO EFFECT
46	49739	890.2	792540.5	59961.5	5765.042	33235705	118178	8464.39	71645862	116858.5	9821.006	96452161	-10913.333	9440.45	-1.156	NO EFFECT
47	50702	1084.0	1175045	61132	5757.263	33146082	119862	8545.45	73024675	118513.5	9908.687	98182085	-11012.5	9518.04	-1.157	NO EFFECT

TABLE B-1 CONTINUED FOR SUB-CMC ALFONIC ENHANCEMENT OF JP-8

Int	Mean Blank Soil O2	Std Dev of Blank Soil O2	Var Blank Soil O2	Mean Sub CMC ALF & Soil O2	Std Dev Sub-CMC ALF & Soil O2	Var Sub-CMC ALF & Soil O2	Mean JP-8 & Soil O2	Std Dev JP-8 & Soil O2	Var JP-8 & Soil O2	Mean JP-8 & Sub-CMC ALF & Soil O2	Std Dev JP-8 & Sub-CMC ALF & Soil O2	Var JP-8 & Sub-CMC ALF & Soil O2	$\bar{X}_{JP8/SURF} - \bar{X}_{JP8} - \bar{X}_{SURF} + \bar{X}_{SOIL}$	Std Dev of Test Statistic	Calc T Val (|T Crit| = 2.447)	Effect of Surfactant Addition
48	51659	1287.6	1658021	62313	5753.021	33097248	121554	8625.16	74393433	120166	9991.419	99828450	-11132.333	9596.34	-1.160	NO EFFECT
49	52618	1511.8	2285522	63509	5761.506	33194952	123226	8703.05	75743012	121804	10087.59	1.02E+08	-11244.333	9688.53	-1.161	NO EFFECT
50	53370	1416.3	2006005	64249	5758.678	33162368	124423	8770.61	76923660	122985	10187.99	1.04E+08	-11315.667	9753.1	-1.160	NO EFFECT
51	54118	1338.6	1791725	65005	5753.021	33097248	125647	8838.98	78127525	124188.5	10290.52	1.06E+08	-11399.5	9820.1	-1.161	NO EFFECT
52	55017	1465.1	2146592	65465	5764.334	33227552	126403	8880.66	78866076	124923	10356.29	1.07E+08	-10891.667	9879.38	-1.102	NO EFFECT
53	55755	1383.8	1914925	66175.5	5765.042	33235705	127570	8948.3	80072126	126049.5	10448.92	1.09E+08	-10962.667	9942.63	-1.103	NO EFFECT
54	56564	1388.1	1926685	66653.5	5777.77	33382621	128336	8991.02	80838522	126802.5	10529.53	1.11E+08	-10641.667	10001.8	-1.064	NO EFFECT
55	57294	1308.9	1713101	67388	5779.891	33407138	129511	9057.68	82041583	127947	10632.06	1.13E+08	-10733	10071.1	-1.066	NO EFFECT
56	57901	1037.3	1076045	67827	5779.891	33407138	130221	9098.88	82789630	128627	10691.45	1.14E+08	-10787.333	10099.1	-1.068	NO EFFECT
57	58627	944.0	891112.5	68544.5	5767.87	33268325	131316	9164.79	83993342	129701	10783.38	1.16E+08	-10865.167	10159.6	-1.069	NO EFFECT
58	59222	688.7	474338	69010	5775.648	33358112	132059	9201.26	84663163	130414	10858.33	1.18E+08	-10946	10202.4	-1.073	NO EFFECT
59	59941	607.4	368940.5	69723.5	5783.426	33448021	133144	9258.38	85717605	131479	10965.81	1.2E+08	-11018.833	10276.5	-1.072	NO EFFECT
60	60662	525.4	276024.5	70155	5792.619	33554432	133830	9297.93	86451534	132135	11030.87	1.22E+08	-10816.667	10323.4	-1.048	NO EFFECT
61	61372	419.3	175824.5	70830	5779.891	33407138	134870	9358.58	87583053	133124.5	11109.35	1.23E+08	-10907.5	10377.6	-1.051	NO EFFECT
62	62070	341.5	116644.5	71524	5777.062	33374450	135925	9420.66	88748858	134133	11194.91	1.25E+08	-11005.333	10439.9	-1.054	NO EFFECT
63	62767	257.4	66248	72204	5764.334	33227552	136939	9476.59	89805692	135102.5	11286.13	1.27E+08	-11091.167	10501	-1.056	NO EFFECT
64	63462	157.0	24642	72876.5	5765.042	33235705	137976	9529.68	90814801	136081	11374.52	1.29E+08	-11198.167	10563.7	-1.060	NO EFFECT
65	64154	58.0	3362	73561.5	5762.213	33203101	139000	9585.99	91891159	137054	11467.86	1.32E+08	-11312.5	10629.7	-1.064	NO EFFECT
66	64846	26.2	684.5	74265.5	5745.243	33007813	140049	9644.68	93019764	138038.5	11557.66	1.34E+08	-11448.667	10691.1	-1.071	NO EFFECT
67	65728	170.4	29040.5	74730	5750.192	33064712	140726	9681.83	93737739	138703.5	11638.27	1.35E+08	-10904.5	10747.9	-1.015	NO EFFECT
68	66451	113.1	12800	75478	5758.678	33162368	141817	9735.73	94784358	139765.5	11755.65	1.38E+08	-10998.833	10829.6	-1.016	NO EFFECT
69	67167	53.0	2812.5	76224.5	5738.172	32926613	142882	9791.83	95879982	140802	11876.57	1.41E+08	-11100.833	10906.5	-1.018	NO EFFECT
70	67869	2.8	8	76977	5734.636	32886050	143973	9843.19	96888402	141850	11979.8	1.44E+08	-11228.667	10977.1	-1.023	NO EFFECT
71	68605	12.7	162	77878	5475.835	29984768	144980	9891.12	97834156	142824	12094.35	1.46E+08	-11437.667	10988.2	-1.041	NO EFFECT
72	69300	79.2	6272	78691.5	5357.748	28705465	146030	9942.48	98852857	143832.5	12202.54	1.49E+08	-11645	11034.4	-1.055	NO EFFECT
73	69999	129.4	16744.5	79517.5	5199.356	27033305	147042	9987.57	99751527	144811	12313.56	1.52E+08	-11841.5	11071.9	-1.070	NO EFFECT
74	70686	190.9	36450	80432.5	4919.342	24199925	148057	10032.6	1.01E+08	145787	12419.62	1.54E+08	-12151.5	11081.2	-1.097	NO EFFECT
75	71368	254.6	64800	81318.5	4674.683	21852661	149071	10081.5	1.02E+08	146759.5	12522.15	1.57E+08	-12441.667	11101.4	-1.121	NO EFFECT
76	72052	309.0	95484.5	82350.5	4219.306	17802545	150078	10129	1.03E+08	147718	12620.44	1.59E+08	-12877.167	11080.9	-1.162	NO EFFECT
77	72869	156.3	24420.5	82954	3996.568	15972552	150709	10157.8	1.03E+08	148308.5	12674.89	1.61E+08	-12596.5	11077.9	-1.137	NO EFFECT
78	73537	204.4	41760.5	83938.5	3582.91	12837245	151695	10203.1	1.04E+08	149225	12733.58	1.62E+08	-13016.833	11055	-1.177	NO EFFECT
79	74330	67.9	4608	84589.5	3341.08	11162813	152352	10232.5	1.05E+08	149849	12797.22	1.64E+08	-12810.167	11062.1	-1.158	NO EFFECT
80	75010	93.3	8712	85599.5	2947.928	8690281	153366	10270.3	1.05E+08	150814.5	12881.36	1.66E+08	-13207.333	11066.8	-1.193	NO EFFECT
81	75773	7.8	60.5	86205	2735.089	7480712	154004	10299.2	1.06E+08	151396	12911.77	1.67E+08	-13035	11066	-1.178	NO EFFECT
82	76434	37.5	1404.5	87133	2382.95	5678450	154947	10345.7	1.07E+08	152294.5	12991.67	1.69E+08	-13378.833	11086.4	-1.207	NO EFFECT
83	77159	16.3	264.5	87746.5	2161.625	4672625	155577	10364.1	1.07E+08	152888.5	13032.69	1.7E+08	-13265.333	11093.5	-1.196	NO EFFECT
84	77817	31.8	1012.5	88631.5	1904.239	3626125	156539	10404.5	1.08E+08	153812.5	13110.47	1.72E+08	-13564	11128.4	-1.219	NO EFFECT
85	78661	194.5	37812.5	89116.5	1810.9	3279361	157112	10426.4	1.09E+08	154335.5	13138.75	1.73E+08	-13095.333	11144.9	-1.175	NO EFFECT
86	79299	126.6	16020.5	89857.5	1676.55	2810821	157993	10463.7	1.09E+08	155157.5	13178.35	1.74E+08	-13305	11168.9	-1.191	NO EFFECT
87	80277	547.3	299538	90320	1630.588	2658818	158583	10485.9	1.1E+08	155720.5	13225.02	1.75E+08	-12518.5	11206.3	-1.117	NO EFFECT
88	80947	526.1	276768	91035.5	1594.526	2542513	159506	10525.3	1.11E+08	156618	13316.23	1.77E+08	-12604.833	11269.4	-1.119	NO EFFECT
89	81617	513.4	263538	91815.5	1450.276	2103301	160412	10556.9	1.11E+08	157498	13406.74	1.8E+08	-12749.167	11322.7	-1.126	NO EFFECT
90	82298	522.6	273060.5	92542	1374.616	1889568	161304	10588.6	1.12E+08	158401	13447.76	1.81E+08	-12778.333	11352.3	-1.126	NO EFFECT
91	83073	668.2	446512.5	93362.5	1322.997	1750321	162228	10614.7	1.13E+08	159451	13327.55	1.78E+08	-12594.167	11290.2	-1.115	NO EFFECT
92	84239	1373.2	1885682	94629.5	1276.328	1629013	163659	10587.2	1.12E+08	161115	13050.36	1.7E+08	-11963.167	11147.9	-1.073	NO EFFECT
93	85249	1852.6	3432200	96098.5	1178.747	1389445	165399	10310.3	1.06E+08	163709	11672.92	1.36E+08	-11229.833	10294.3	-1.091	NO EFFECT
94	86260	2343.4	5491298	97569.5	1120.764	1256113	167137	10114	1.02E+08	165881.5	10946.72	1.2E+08	-10908.333	9868.46	-1.105	NO EFFECT
95	87269	2838.3	8056098	99078.5	1014.698	1029613	168828	9969.6	99392943	167695.5	10707.72	1.15E+08	-10935	9746.87	-1.122	NO EFFECT

TABLE B-2 DATA FOR DETERMINING CMC ALFONIC ENHANCEMENT OF JP-8 BIODEGRADATION

Int	Mean Blank Soil O2	Std Dev of Blank Soil O2	Var Blank Soil O2	Mean Sub CMC ALF & Soil O2	Std Dev Sub-CMC ALF & Soil O2	Var Sub-CMC ALF & Soil O2	Mean JP-8 & Soil O2	Std Dev JP-8 & Soil O2	Var JP-8 & Soil O2	Mean JP-8 & Sub-CMC ALF & Soil O2	Std Dev JP-8 & Sub-CMC ALF & Soil O2	Var JP-8 & Sub-CMC ALF & Soil O2	$\bar{X}_{JP8/SURF} - \bar{X}_{JP8} - \bar{X}_{SURF} + \bar{X}_{SOIL}$	Std Dev of Test Statistic	Calc T Val ($\lvert T_{Crit} \rvert = 2.447$)	Effect of Surfactant Addition
1	1687	225.6	50880.5	2703	169.0	28561	3608	330.4	109192	4623	268.7	72200	-160.2	327.8	-0.489	NO EFFECT
2	3292	371.2	137812.5	5239	309.0	95485	7262	529.3	280147	9335	655.5	429665	-137.0	639.5	-0.214	NO EFFECT
3	4814	468.1	219122	7579	437.0	190962	10918	749.4	561541	13975	1004.1	1008200	-39.0	929.8	-0.042	NO EFFECT
4	6264	552.3	304980.5	9752	554.4	307328	14650	991.9	983820	18554	1250.2	1562912	25.3	1168.0	0.022	NO EFFECT
5	7678	627.9	394272	11792	668.9	447458	18395	1246.7	1554220	23057	1463.7	2142450	104.3	1391.3	0.075	NO EFFECT
6	9041	694.4	482162	13695	772.9	597325	22112	1516.8	2300716	27430	1641.2	2693521	173.3	1598.1	0.108	NO EFFECT
7	10364	758.0	574592	15508	874.7	765085	25821	1792.4	3212850	31729	1781.9	3175200	228.2	1789.1	0.128	NO EFFECT
8	11649	823.8	678612.5	17229	975.1	950821	29458	2066.8	4271557	35899	1883.7	3548448	278.2	1963.2	0.142	NO EFFECT
9	12886	869.7	756450	18873	1069.1	1143072	33019	2340.1	5476188	39991	1966.5	3866981	369.5	2125.6	0.174	NO EFFECT
10	14080	917.8	842402	20441	1156.1	1336613	36468	2614.0	6833257	43944	2016.0	4064101	465.7	2275.2	0.205	NO EFFECT
11	15227	946.1	895122	21951	1244.5	1548800	39811	2884.1	8318006	47804	2049.2	4199202	599.7	2415.8	0.248	NO EFFECT
12	16437	900.1	810264.5	23420	1329.4	1767200	43049	3112.2	9685790	51582	2050.6	4205000	912.7	2515.0	0.363	NO EFFECT
13	17554	937.6	879138	24851	1412.8	1996002	46255	3377.9	11409994	55276	2039.3	4158728	1061.3	2643.4	0.402	NO EFFECT
14	18645	980.8	961884.5	26233	1483.5	2200802	49352	3638.1	13235611	58860	2012.4	4049858	1226.0	2766.1	0.443	NO EFFECT
15	19708	996.3	992640.5	27602	1562.0	2439841	52403	3893.1	15155977	62402	1983.4	3934013	1400.0	2885.9	0.485	NO EFFECT
16	20756	1018.2	1036800	28938	1633.4	2668050	55361	4135.4	17101842	65850	1937.5	3753800	1586.7	2997.5	0.529	NO EFFECT
17	21769	1014.0	1028178	30239	1695.6	2875202	58258	4372.4	19117933	69220	1877.4	3524513	1774.5	3099.6	0.572	NO EFFECT
18	22786	1032.4	1065800	31520	1751.5	3067765	61075	4599.3	21153121	72525	1811.6	3281922	1986.2	3201.2	0.620	NO EFFECT
19	23755	1005.5	1011042	32776	1798.9	3235968	63813	4819.9	23231780	75733	1745.1	3045512	2188.3	3294.1	0.664	NO EFFECT
20	24736	1020.4	1041125	34017	1840.6	3387805	66476	5032.9	25330066	78856	1672.3	2796613	2376.7	3389.9	0.701	NO EFFECT
21	25695	1033.1	1067261	35231	1877.4	3524513	69039	5236.2	27417703	81876	1596.6	2549282	2570.5	3481.7	0.738	NO EFFECT
22	26638	1047.2	1096681	36433	1910.6	3650402	71529	5435.4	29543908	84823	1528.8	2337122	2757.7	3575.1	0.771	NO EFFECT
23	27575	1067.7	1140050	37624	1949.5	3800525	73949	5629.3	31688760	87683	1466.5	2150738	2930.8	3670.9	0.798	NO EFFECT
24	28509	1087.5	1182722	38786	1977.1	3908808	76287	5816.8	33835711	90449	1400.1	1960200	3116.0	3762.0	0.828	NO EFFECT
25	29424	1140.6	1300885	39924	1974.2	3897632	78490	5992.6	35911092	93094	1338.6	1791725	3296.2	3849.1	0.856	NO EFFECT
26	30339	1205.6	1453513	41030	2015.3	4061250	80624	6156.2	37898413	95661	1288.3	1659842	3493.0	3942.5	0.886	NO EFFECT
27	31263	1272.1	1618201	42146	2057.7	4234050	82713	6325.2	40007844	98182	1231.8	1517282	3686.0	4039.2	0.913	NO EFFECT
28	32176	1344.9	1808802	43237	2102.2	4419365	84711	6487.0	42080577	100599	1176.6	1384448	3876.8	4134.8	0.938	NO EFFECT
29	33080	1407.1	1980050	44324	2144.7	4599545	86689	6643.1	44130820	102997	1127.8	1272013	4069.3	4227.2	0.963	NO EFFECT
30	33976	1460.2	2132113	45405	2182.8	4764785	88604	6792.2	46133587	105322	1082.6	1171981	4256.0	4314.9	0.986	NO EFFECT
31	34865	1491.3	2223941	46480	2222.4	4939225	90461	6932.5	48059037	107593	1038.7	1078981	4462.0	4395.2	1.015	NO EFFECT
32	35738	1496.9	2240845	47548	2259.9	5107208	92321	7067.9	49954610	109855	993.5	987013	4664.8	4468.5	1.044	NO EFFECT
33	36615	1519.6	2309101	48617	2301.6	5297513	94132	7196.9	51795820	112063	953.2	908552	4854.8	4543.1	1.069	NO EFFECT
34	37470	1524.5	2324168	49665	2333.5	5445000	95893	7327.7	53694580	114240	922.8	851513	5073.2	4615.3	1.099	NO EFFECT
35	38337	1533.7	2352281	50732	2372.3	5628013	97644	7447.3	55463004	116374	888.8	790025	5250.0	4683.5	1.121	NO EFFECT
36	39236	1519.6	2309101	51814	2407.7	5797013	99307	7562.1	57185144	117771	35.4	1250	4811.2	4706.3	1.022	NO EFFECT
37	40224	1400.1	1960200	53050	2458.6	6044765	101101	7667.8	58795075	119929	60.1	3613	5012.0	4753.4	1.054	NO EFFECT
38	41276	1193.6	1424672	54412	2512.4	6311905	102979	7763.4	60270633	122168	83.4	6962	5209.8	4786.4	1.088	NO EFFECT
39	42368	893.1	797584.5	55871	2561.8	6563065	104947	7854.2	61689112	124473	84.9	7200	5391.8	4811.8	1.121	NO EFFECT
40	43542	623.0	388080.5	57387	2617.0	6848701	107002	7948.3	63175500	126839	90.5	8192	5551.2	4851.8	1.144	NO EFFECT
41	44698	371.9	138338	58864	2670.7	7132865	109018	8041.5	64665163	129147	96.2	9248	5700.5	4899.6	1.163	NO EFFECT
42	45757	19.8	392	60309	2723.1	7415101	111024	8132.0	66129194	131425	78.5	6161	5834.7	4951.6	1.178	NO EFFECT
43	46783	270.1	72962	61683	2771.9	7683200	112940	8224.0	67634990	133611	58.7	3445	5961.8	5014.4	1.189	NO EFFECT
44	47772	512.7	262812.5	62973	2821.4	7960050	114741	8305.9	68988761	135693	41.7	1741	6112.2	5077.6	1.204	NO EFFECT
45	48766	702.2	493024.5	64228	2870.9	8241800	116480	8386.4	70331436	137702	22.6	512	6255.7	5141.8	1.217	NO EFFECT
46	49739	890.2	792540.5	65455	2914.7	8495442	118178	8464.4	71645862	139673	4.2	18	6407.7	5206.7	1.231	NO EFFECT
47	50702	1084.0	1175045	66667	2957.8	8748745	119862	8545.4	73024675	141628	24.7	613	6567.0	5276.9	1.244	NO EFFECT

TABLE B-2 CONTINUED FOR CMC ALFONIC ENHANCEMENT OF JP-8

Int	Mean Blank Soil O2	Std Dev of Blank Soil O2	Var Blank Soil O2	Mean Sub CMC ALF & Soil O2	Std Dev Sub-CMC ALF & Soil O2	Var Sub-CMC ALF & Soil O2	Mean JP-8 & Soil O2	Std Dev JP-8 & Soil O2	Var JP-8 & Soil O2	Mean JP-8 & Sub-CMC ALF & Soil O2	Std Dev JP-8 & Sub-CMC ALF & Soil O2	Var JP-8 & Sub-CMC ALF & Soil O2	$\bar{X}_{JP8/SURF} - \bar{X}_{JP8} - \bar{X}_{SURF} + \bar{X}_{SOIL}$	Std Dev of Test Statistic	Calc T Val (|T Crit| = 2.447)	Effect of Surfactant Addition
48	51659	1287.6	1658021	67880	3001.7	9010013	121554	8625.2	74393433	143569	46.0	2113	6703.7	5350.8	1.253	NO EFFECT
49	52618	1511.8	2285522	69105	3052.6	9318245	123226	8703.0	75743012	145505	62.9	3961	6860.7	5431.3	1.263	NO EFFECT
50	53370	1416.3	2006005	69903	3086.5	9526613	124423	8770.6	76923660	146981	81.3	6613	7026.3	5461.0	1.287	NO EFFECT
51	54118	1338.6	1791725	70717	3121.2	9741698	125647	8839.0	78127525	148499	103.2	10658	7199.0	5494.6	1.310	NO EFFECT
52	55017	1465.1	2146592	71211	3144.5	9887905	126403	8880.7	78866076	149428	113.1	12800	7867.8	5537.5	1.421	NO EFFECT
53	55755	1383.8	1914925	71998	3184.1	10138505	127570	8948.3	80072126	150877	134.4	18050	8042.8	5571.1	1.444	NO EFFECT
54	56564	1388.1	1926685	72508	3207.4	10287648	128336	8991.0	80838522	151824	146.4	21425	8524.8	5599.1	1.523	NO EFFECT
55	57294	1308.9	1713101	73303	3247.0	10543232	129511	9057.7	82041583	153294	173.9	30258	8699.0	5633.2	1.544	NO EFFECT
56	57901	1037.3	1076045	73767	3270.4	10695313	130221	9098.9	82789630	154168	190.2	36181	8813.7	5631.9	1.565	NO EFFECT
57	58627	944.0	891112.5	74527	3305.7	10927813	131316	9164.8	83993342	155580	212.1	45000	9031.8	5666.4	1.594	NO EFFECT
58	59222	688.7	474338	75015	3331.2	11096761	132059	9201.3	84663163	156472	229.8	52813	9107.0	5673.1	1.605	NO EFFECT
59	59941	607.4	368940.5	75778	3367.2	11338322	133144	9258.4	85717605	157862	252.4	63725	9309.2	5706.9	1.631	NO EFFECT
60	60662	525.4	276024.5	76234	3391.3	11500808	133830	9297.9	86451534	158707	267.3	71442	9676.3	5729.3	1.689	NO EFFECT
61	61372	419.3	175824.5	76956	3424.5	11727325	134870	9358.6	87583053	160025	282.8	80000	9867.5	5764.7	1.712	NO EFFECT
62	62070	341.5	116644.5	77691	3460.6	11975618	135925	9420.7	88748858	161339	289.9	84050	10033.7	5803.0	1.729	NO EFFECT
63	62767	257.4	66248	78401	3492.4	12196861	136939	9476.6	89805692	162629	312.5	97682	10238.8	5838.0	1.754	NO EFFECT
64	63462	157.0	24642	79108	3522.8	12410162	137976	9529.7	90814801	163918	333.8	111392	10407.3	5871.7	1.772	NO EFFECT
65	64154	58.0	3362	79821	3553.2	12625313	139000	9586.0	91891159	165208	347.2	120541	10582.0	5907.7	1.791	NO EFFECT
66	64846	26.2	684.5	80552	3586.4	12862592	140049	9644.7	93019764	166507	357.8	128018	10733.3	5946.3	1.805	NO EFFECT
67	65728	170.4	29040.5	81026	3609.8	13030513	140726	9681.8	93737739	167352	366.3	134162	11448.5	5972.5	1.917	NO EFFECT
68	66451	113.1	12800	81806	3648.7	13312800	141817	9735.7	94784358	168739	376.2	141512	11646.7	6009.1	1.938	NO EFFECT
69	67167	53.0	2812.5	82569	3680.5	13546013	142882	9791.8	95879982	170079	385.4	148513	11831.7	6045.7	1.957	NO EFFECT
70	67869	2.8	8	83338	3721.5	13849585	143973	9843.2	96888402	171435	389.6	151801	11995.3	6081.8	1.972	NO EFFECT
71	68605	12.7	162	84065	3744.8	14023808	144980	9891.1	97834156	172719	398.1	158485	12269.8	6112.7	2.007	NO EFFECT
72	69300	79.2	6272	84805	3780.2	14289858	146030	9942.5	98852857	174023	403.8	163021	12431.5	6148.1	2.022	NO EFFECT
73	69999	129.4	16744.5	85537	3810.6	14520661	147042	9987.6	99751527	175297	410.1	168200	12625.5	6179.3	2.043	NO EFFECT
74	70686	190.9	36450	86268	3841.7	14758745	148057	10032.6	100652524	176569	415.8	172872	12795.5	6210.9	2.060	NO EFFECT
75	71368	254.6	64800	86990	3872.1	14993288	149071	10081.5	101637329	177826	422.8	178802	12953.3	6244.9	2.074	NO EFFECT
76	72052	309.0	95484.5	87714	3899.7	15207613	150078	10129.0	102596122	179074	426.4	181805	13115.3	6277.5	2.089	NO EFFECT
77	72869	156.3	24420.5	88154	3916.0	15334722	150709	10157.8	103181776	179836	429.2	184225	13730.5	6293.6	2.182	NO EFFECT
78	73537	204.4	41760.5	88860	3935.8	15490178	151695	10203.1	104102964	181046	424.3	180000	13882.7	6322.6	2.196	NO EFFECT
79	74330	67.9	4608	89327	3951.3	15612872	152352	10232.5	104705029	181839	427.1	182408	14442.3	6340.3	2.278	NO EFFECT
80	75010	93.3	8712	90073	3974.6	15797821	153366	10270.3	105479616	183109	393.9	155125	14613.7	6364.6	2.296	NO EFFECT
81	75773	7.8	60.5	90521	3987.4	15899161	154004	10299.2	106074381	183877	386.8	149605	15130.0	6382.2	2.371	NO EFFECT
82	76434	37.5	1404.5	91214	4007.2	16057445	154947	10345.7	107032686	185190	175.4	30752	15436.2	6406.7	2.409	NO EFFECT
83	77159	16.3	264.5	91659	4020.6	16165298	155577	10364.1	107414158	185978	119.5	14281	15911.2	6418.8	2.479	ENHANCED
84	77817	31.8	1012.5	92363	4044.7	16359200	156539	10404.5	108253621	187485	340.8	116162	16377.0	6449.5	2.539	ENHANCED
85	78661	194.5	37812.5	92771	4056.0	16450848	157112	10426.4	108709177	188248	447.6	200345	17162.2	6468.3	2.653	ENHANCED
86	79299	126.6	16020.5	93417	4075.8	16611848	157993	10463.7	109488169	189595	815.3	664705	17572.5	6509.5	2.700	ENHANCED
87	80277	547.3	299538	93838	4093.4	16756261	158583	10485.9	109954156	190383	932.7	869881	18626.0	6543.8	2.846	ENHANCED
88	80947	526.1	276768	94501	4118.2	16959488	159506	10525.3	110782722	191882	1480.7	2192418	19193.7	6619.3	2.900	ENHANCED
89	81617	513.4	263538	95165	4134.5	17093705	160412	10556.9	111447736	193340	1981.3	3925602	19743.8	6703.9	2.945	ENHANCED
90	82298	522.6	273060.5	95836	4152.1	17240192	161304	10588.6	112117676	194742	2422.5	5868738	20268.7	6796.3	2.982	ENHANCED
91	83073	668.2	446512.5	96680	4162.0	17322498	162228	10614.7	112672636	196139	2752.1	7573832	20776.3	6880.5	3.020	ENHANCED
92	84239	1373.2	1885682	97910	4186.1	17523200	163659	10587.2	112089350	197854	2878.6	8286521	21494.8	6949.1	3.093	ENHANCED
93	85249	1852.6	3432200	99294	4205.2	17683405	165399	10310.3	106302034	199688	2967.7	8807405	21553.7	6888.3	3.129	ENHANCED
94	86260	2343.4	5491298	100702	4222.8	17832392	167137	10114.0	102292146	201848	2589.4	6705122	21925.7	6792.6	3.228	ENHANCED
95	87269	2838.3	8056098	102183	4346.6	18892805	168828	9969.6	99392943	203780	2491.1	6205765	22045.0	6823.5	3.231	ENHANCED

TABLE B-3 DATA FOR DETERMINING SUPRA-CMC ALFONIC ENHANCEMENT OF JP-8 BIODEGRADATION

Int	Mean Blank Soil O2	Std Dev of Blank Soil O2	Var Blank Soil O2	Mean Sub CMC ALF & Soil O2	Std Dev Sub-CMC ALF & Soil O2	Var Sub-CMC ALF & Soil O2	Mean JP-8 & Soil O2	Std Dev JP-8 & Soil O2	Var JP-8 & Soil O2	Mean JP-8 & Sub-CMC ALF & Soil O2	Std Dev JP-8 & Sub-CMC ALF & Soil O2	Var JP-8 & Sub-CMC ALF & Soil O2	$\bar{X}_{JP8/SURF} - \bar{X}_{JP8} - \bar{X}_{SURF} + \bar{X}_{SOIL}$	Std Dev of Test Statistic	Calc T Val (|T Crit| = 2.447)	Effect of Surfactant Addition
1	1687	225.6	50881	4655	70.7	5000	3608	330.4	109192	4571	170.4	29041	-2165.2	279.3	-7.751	INHIBITED
2	3292	371.2	137813	10532	33.9	1152	7262	529.3	280147	10622	376.9	142045	-4143.5	483.4	-8.571	INHIBITED
3	4814	468.1	219122	15851	206.5	42632	10918	749.4	561541	16204	582.7	339488	-6082.0	693.3	-8.772	INHIBITED
4	6264	552.3	304981	20696	439.1	192821	14650	991.9	983820	21509	816.0	665858	-7963.2	936.8	-8.500	INHIBITED
5	7678	627.9	394272	25281	688.7	474338	18395	1246.7	1554220	26738	1086.8	1181185	-9704.2	1209.9	-8.020	INHIBITED
6	9041	694.4	482162	29719	944.7	892448	22112	1516.8	2300716	31971	1347.7	1816418	-11309.7	1487.8	-7.601	INHIBITED
7	10364	758.0	574592	34075	1209.9	1463761	25821	1792.4	3212850	37335	1599.5	2558322	-12732.8	1767.9	-7.202	INHIBITED
8	11649	823.8	678613	38292	1468.0	2154888	29458	2066.8	4271557	42777	1793.9	3218185	-13907.8	2022.5	-6.877	INHIBITED
9	12886	869.7	756450	42315	1709.8	2923362	33019	2340.1	5476188	48265	1968.6	3875328	-14798.0	2261.8	-6.543	INHIBITED
10	14080	917.8	842402	46055	1933.2	3737378	36468	2614.0	6833257	53660	2111.4	4458098	-15432.3	2484.7	-6.211	INHIBITED
11	15227	946.1	895122	49490	2153.8	4639058	39811	2884.1	8318006	58892	2231.6	4980168	-15851.3	2693.8	-5.884	INHIBITED
12	16437	900.1	810265	52631	2370.9	5621305	43049	3112.2	9685790	63896	2299.5	5287752	-15983.8	2855.1	-5.598	INHIBITED
13	17554	937.6	879138	55482	2571.7	6613885	46255	3377.9	11409994	68697	2353.3	5537792	-16148.2	3035.9	-5.319	INHIBITED
14	18645	980.8	961885	58080	2770.4	7675362	49352	3638.1	13235611	73230	2374.5	5638082	-16251.0	3204.7	-5.071	INHIBITED
15	19708	996.3	992641	60489	2947.2	8686112	52403	3893.1	15155977	77560	2376.6	5648161	-16329.5	3356.7	-4.865	INHIBITED
16	20756	1018.2	1036800	62736	3116.2	9710825	55361	4135.4	17101842	81679	2389.3	5708821	-16382.3	3508.6	-4.669	INHIBITED
17	21769	1014.0	1028178	64853	3263.3	10649113	58258	4372.4	19117933	85591	2387.9	5702065	-16468.0	3645.2	-4.518	INHIBITED
18	22786	1032.4	1065800	66876	3404.7	11592113	61075	4599.3	21153121	89345	2393.6	5729113	-16550.3	3783.2	-4.375	INHIBITED
19	23755	1005.5	1011042	68804	3534.1	12490002	63813	4819.9	23231780	92940	2414.8	5831113	-16633.2	3915.1	-4.248	INHIBITED
20	24736	1020.4	1041125	70663	3648.0	13307641	66476	5032.9	25330066	96384	2437.4	5940905	-16741.3	4046.0	-4.138	INHIBITED
21	25695	1033.1	1067261	72455	3754.0	14092741	69039	5236.2	27417703	99689	2450.8	6006578	-16840.5	4168.2	-4.040	INHIBITED
22	26638	1047.2	1096681	74198	3860.1	14900341	71529	5435.4	29543908	102881	2461.4	6058681	-16949.3	4288.6	-3.952	INHIBITED
23	27575	1067.7	1140050	75885	3957.0	15657608	73949	5629.3	31688760	105962	2469.9	6100525	-17052.2	4404.8	-3.871	INHIBITED
24	28509	1087.5	1182722	77532	4049.6	16399265	76287	5816.8	33835711	108946	2489.7	6198721	-17133.0	4520.6	-3.790	INHIBITED
25	29424	1140.6	1300885	79119	4150.7	17228450	78490	5992.6	35911092	111818	2504.6	6272882	-17174.3	4636.8	-3.704	INHIBITED
26	30339	1205.6	1453513	80659	4237.0	17952032	80624	6156.2	37898413	114573	2514.5	6322568	-17224.0	4743.9	-3.631	INHIBITED
27	31263	1272.1	1618201	82197	4326.8	18721081	82713	6325.2	40007844	117289	2525.1	6376021	-17258.0	4855.2	-3.555	INHIBITED
28	32176	1344.9	1808802	83685	4416.6	19506258	84711	6487.0	42080577	119915	2530.0	6401042	-17255.7	4963.3	-3.477	INHIBITED
29	33080	1407.1	1980050	85159	4507.1	20313938	86689	6643.1	44130820	122495	2537.8	6440461	-17268.2	5068.7	-3.407	INHIBITED
30	33976	1460.2	2132113	86608	4596.2	21125000	88604	6792.2	46133587	125013	2544.2	6472802	-17256.0	5169.3	-3.338	INHIBITED
31	34865	1491.3	2223941	88046	4682.5	21925442	90461	6932.5	48059037	127486	2551.2	6508832	-17211.0	5262.6	-3.270	INHIBITED
32	35738	1496.9	2240845	89469	4772.3	22774501	92321	7067.9	49954610	129933	2563.3	6570313	-17177.7	5352.4	-3.209	INHIBITED
33	36615	1519.6	2309101	90875	4855.0	23570978	94132	7196.9	51795820	132337	2568.2	6595712	-17129.7	5438.3	-3.150	INHIBITED
34	37470	1524.5	2324168	92252	4957.5	24577061	95893	7327.7	53694580	134668	2537.8	6440461	-17085.3	5520.2	-3.095	INHIBITED
35	38337	1533.7	2352281	93628	5054.4	25546952	97644	7447.3	55463004	136958	2560.4	6555821	-17062.5	5608.7	-3.042	INHIBITED
36	39236	1519.6	2309101	95009	5149.2	26513762	99307	7562.1	57185144	139196	2537.8	6440461	-16959.8	5681.1	-2.985	INHIBITED
37	40224	1400.1	1960200	96553	5227.6	27328225	101101	7667.8	58795075	141587	2503.2	6265800	-16832.5	5728.9	-2.938	INHIBITED
38	41276	1193.6	1424672	98220	5291.3	27997645	102979	7763.4	60270633	144078	2478.4	6142513	-16688.7	5762.5	-2.896	INHIBITED
39	42368	893.1	797585	99986	5347.8	28599485	104947	7854.2	61689112	146634	2448.0	5992722	-16562.2	5787.2	-2.862	INHIBITED
40	43542	623.0	388081	101812	5386.0	29009345	107002	7948.3	63175500	149246	2420.4	5858465	-16467.3	5818.2	-2.830	INHIBITED
41	44698	371.9	138338	103600	5427.8	29460488	109018	8041.5	64665163	151794	2390.7	5715581	-16389.5	5856.8	-2.798	INHIBITED
42	45757	19.8	392	105352	5465.9	29876450	111024	8132.0	66129194	154308	2361.7	5577800	-16325.3	5898.4	-2.768	INHIBITED
43	46783	270.1	72962	107035	5506.9	30326472	112940	8224.0	67634990	156732	2325.7	5408761	-16269.2	5949.3	-2.735	INHIBITED
44	47772	512.7	262813	108637	5553.6	30842658	114741	8305.9	68988761	159047	2279.7	5197088	-16197.3	6000.6	-2.699	INHIBITED
45	48766	702.2	493025	110199	5603.1	31394888	116480	8386.4	70331436	161300	2229.5	4970705	-16117.8	6053.2	-2.663	INHIBITED
46	49739	890.2	792541	111733	5657.6	32008001	118178	8464.4	71645862	163514	2169.4	4706312	-16028.8	6107.4	-2.624	INHIBITED
47	50702	1084.0	1175045	113253	5717.0	32683613	119862	8545.4	73024675	165694	2121.3	4500000	-15952.5	6170.4	-2.585	INHIBITED

TABLE B-3 CONTINUED FOR SUPRA-CMC ALFONIC ENHANCEMENT OF JP-8

Int	Mean Blank Soil O2	Std Dev of Blank Soil O2	Var Blank Soil O2	Mean Sub CMC ALF & Soil O2	Std Dev Sub-CMC ALF & Soil O2	Var Sub-CMC ALF & Soil O2	Mean JP-8 & Soil O2	Std Dev JP-8 & Soil O2	Var JP-8 & Soil O2	Mean JP-8 & Sub-CMC ALF & Soil O2	Std Dev JP-8 & Sub-CMC ALF & Soil O2	Var JP-8 & Sub-CMC ALF & Soil O2	$\bar{X}_{JP8/SURF} - \bar{X}_{JP8} - \bar{X}_{SURF} + \bar{X}_{SOIL}$	Std Dev of Test Statistic	Calc T Val (|T Crit| = 2.447)	Effect of Surfactant Addition
48	51659	1287.6	1658021	114768	5777.8	33382621	121554	8625.2	74393433	167857	2066.2	4269042	-15895.8	6236.1	-2.549	INHIBITED
49	52618	1511.8	2285522	116295	5838.6	34089025	123226	8703.0	75743012	170010	2012.4	4049858	-15823.8	6307.0	-2.509	INHIBITED
50	53370	1416.3	2006005	117356	5905.8	34877952	124423	8770.6	76923660	170347	132.2	17485	-17061.2	6187.0	-2.758	INHIBITED
51	54118	1338.6	1791725	118433	5975.1	35701250	125647	8839.0	78127525	172001	87.7	7688	-17015.0	6232.4	-2.730	INHIBITED
52	55017	1465.1	2146592	119080	6031.6	36380450	126403	8880.7	78866076	173006	52.3	2738	-16423.7	6284.1	-2.614	INHIBITED
53	55755	1383.8	1914925	120108	6125.0	37515122	127570	8948.3	80072126	174576	4.2	18	-16368.7	6336.7	-2.583	INHIBITED
54	56564	1388.1	1926685	120781	6170.2	38071538	128336	8991.0	80838522	175593	31.8	1013	-15979.2	6371.9	-2.508	INHIBITED
55	57294	1308.9	1713101	121841	6243.8	38984450	129511	9057.7	82041583	177166	78.5	6161	-15967.5	6418.9	-2.488	INHIBITED
56	57901	1037.3	1076045	122454	6316.6	39899245	130221	9098.9	82789630	178102	110.3	12168	-15938.8	6437.4	-2.476	INHIBITED
57	58627	944.0	891113	123452	6413.5	41132450	131316	9164.8	83993342	179604	159.1	25313	-15870.2	6493.6	-2.444	INHIBITED
58	59222	688.7	474338	124105	6462.2	41760661	132059	9201.3	84663163	180553	192.3	36992	-15901.5	6511.3	-2.442	INHIBITED
59	59941	607.4	368941	125134	6541.4	42790501	133144	9258.4	85717605	182030	248.9	61952	-15877.8	6561.4	-2.420	INHIBITED
60	60662	525.4	276025	125741	6605.1	43627141	133830	9297.9	86451534	182925	285.0	81205	-15612.7	6598.3	-2.366	INHIBITED
61	61372	419.3	175825	126709	6708.3	45001585	134870	9358.6	87583053	184309	347.9	121032	-15601.5	6659.1	-2.343	NO EFFECT
62	62070	341.5	116645	127692	6812.3	46406978	135925	9420.7	88748858	185691	416.5	173461	-15615.8	6722.9	-2.323	NO EFFECT
63	62767	257.4	66248	128660	6916.2	47833981	136939	9476.6	89805692	187050	484.4	234613	-15599.7	6784.6	-2.299	NO EFFECT
64	63462	157.0	24642	129620	7017.3	49242888	137976	9529.7	90814801	188399	544.5	296450	-15623.7	6844.4	-2.283	NO EFFECT
65	64154	58.0	3362	130579	7126.9	50793121	139000	9586.0	91891159	189748	619.4	383688	-15635.5	6910.5	-2.263	NO EFFECT
66	64846	26.2	685	131553	7231.6	52295765	140049	9644.7	93019764	191104	689.4	475313	-15670.7	6976.9	-2.246	NO EFFECT
67	65728	170.4	29041	132202	7286.7	53096513	140726	9681.8	93737739	191982	729.7	532512	-15097.5	7016.1	-2.152	NO EFFECT
68	66451	113.1	12800	133264	7378.0	54434178	141817	9735.7	94784358	193412	799.7	639581	-15138.8	7075.7	-2.140	NO EFFECT
69	67167	53.0	2813	134311	7462.1	55682905	142882	9791.8	95879982	194803	873.3	762613	-15186.3	7134.7	-2.129	NO EFFECT
70	67869	2.8	8	135352	7551.2	57020521	143973	9843.2	96888402	196191	936.2	876488	-15262.2	7193.1	-2.122	NO EFFECT
71	68605	12.7	162	136369	7636.0	58309201	144980	9891.1	97834156	197525	1011.2	1022450	-15227.2	7249.8	-2.100	NO EFFECT
72	69300	79.2	6272	137387	7714.5	59514050	146030	9942.5	98852857	198858	1081.9	1170450	-15315.0	7306.0	-2.096	NO EFFECT
73	69999	129.4	16745	138396	7799.4	60830450	147042	9987.6	99751527	200166	1155.4	1334978	-15365.0	7362.3	-2.087	NO EFFECT
74	70686	190.9	36450	139392	7887.8	62217013	148057	10032.6	100652524	201469	1224.0	1498181	-15429.0	7420.1	-2.079	NO EFFECT
75	71368	254.6	64800	140387	7965.6	63450113	149071	10081.5	101637329	202752	1296.8	1681778	-15517.2	7476.8	-2.075	NO EFFECT
76	72052	309.0	95485	141422	8100.6	65619968	150078	10129.0	102596122	204029	1370.4	1877922	-15637.7	7553.7	-2.070	NO EFFECT
77	72869	156.3	24421	142068	8237.8	67861250	150709	10157.8	103181776	204815	1422.0	2022061	-15204.5	7618.2	-1.996	NO EFFECT
78	73537	204.4	41761	143088	8442.1	71269861	151695	10203.1	104102964	206055	1513.9	2291941	-15336.3	7721.7	-1.986	NO EFFECT
79	74330	67.9	4608	143759	8532.7	72806245	152352	10232.5	104705029	206869	1561.3	2437632	-14959.2	7771.2	-1.925	NO EFFECT
80	75010	93.3	8712	144824	8671.3	75190585	153366	10270.3	105479616	208143	1645.4	2707465	-15103.3	7847.4	-1.925	NO EFFECT
81	75773	7.8	61	145457	8785.8	77190313	154004	10299.2	106074381	208927	1698.5	2884802	-14755.5	7907.6	-1.866	NO EFFECT
82	76434	37.5	1405	146469	9006.4	81115585	154947	10345.7	107032686	210126	1793.9	3218185	-14883.3	8020.3	-1.856	NO EFFECT
83	77159	16.3	265	147134	9121.7	83205000	155577	10364.1	107414158	210894	1844.8	3403441	-14647.8	8077.2	-1.813	NO EFFECT
84	77817	31.8	1013	148249	9410.2	88551432	156539	10404.5	108253621	212093	1921.9	3693762	-14901.0	8212.7	-1.814	NO EFFECT
85	78661	194.5	37813	148871	9569.3	91571045	157112	10426.4	108709177	212799	1979.2	3917201	-14386.3	8290.8	-1.735	NO EFFECT
86	79299	126.6	16021	149949	9951.1	99024665	157993	10463.7	109488169	213908	2072.5	4295381	-14646.0	8465.2	-1.730	NO EFFECT
87	80277	547.3	299538	150607	10091.1	101830721	158583	10485.9	109954156	214619	2119.9	4494002	-13906.5	8543.5	-1.628	NO EFFECT
88	80947	526.1	276768	151787	10513.3	110528712	159506	10525.3	110782722	215747	2202.6	4851613	-14227.8	8737.0	-1.628	NO EFFECT
89	81617	513.4	263538	153240	11322.9	128208085	160412	10556.9	111447736	216869	2283.2	5213221	-14802.7	9089.8	-1.629	NO EFFECT
90	82298	522.6	273061	154620	12035.7	144857221	161304	10588.6	112117676	217974	2361.7	5577800	-15282.8	9411.9	-1.624	NO EFFECT
91	83073	668.2	446513	156001	12566.0	157904221	162228	10614.7	112672636	219171	2439.5	5951250	-15512.2	9663.9	-1.605	NO EFFECT
92	84239	1373.2	1885682	157590	12745.6	162450313	163659	10587.2	112089350	221034	2795.9	7817058	-15004.2	9816.5	-1.528	NO EFFECT
93	85249	1852.6	3432200	159274	12848.8	165092621	165399	10310.3	106302034	223405	2100.8	4413421	-14709.3	9715.3	-1.514	NO EFFECT
94	86260	2343.4	5491298	160946	12907.5	166604258	167137	10114.0	102292146	225634	1646.9	2712121	-14532.8	9681.6	-1.501	NO EFFECT
95	87269	2838.3	8056098	162591	12940.8	167463301	168828	9969.6	99392943	227839	1210.6	1465472	-14303.5	9680.5	-1.478	NO EFFECT

Appendix C STATISTICAL DATA FOR DETERMINING IF BIODEGRADATION OF JP-8 OCCURRED

The following table summarizes the data for the uncontaminated and JP-8 contaminated Kittyhawk Silt soil microcosms. The data summaries the mean, standard deviation, and upper and lower 95% confidence intervals for the oxygen uptake, for each of the treatments. The values for statistical hypothesis difference of the sample means, and comparison of the calculated test statistic (t) to the critical t value using a 95% confidence level is given also. The ultimate determination of no effect, biodegradation, or inhibition is listed in the last column for each interval sampled.

<u>List of Tables</u>

TABLE C-1 DATA FOR DETERMINING JP-8 DEGRADATION

Time (Hrs)	MEAN Blank Soil O2	STD DEV	STD ERROR	UPPER 95% C.I. Blank Soil O2	LOWER 95% C.I. Blank Soil O2	MEAN JP-8 & Soil O2	STD DEV	STD ERROR	UPPER 95% C.I. JP-8 & Soil O2	LOWER 95% C.I. JP-8 & Soil O2	$\overline{X}_{JP8} - \overline{X}_{SOIL}$	Calc T Val (|T Crit| = 2.228)	DEGRADATION/ INHIBITION/ NO EFFECT
4.1	1709	233.169466	104.2765554	1998.519	1419.481	3340	572.789083	216.4939	3869.742	2810.258065			
10.4	3400.575	327.694552	146.5494588	3807.463	2993.688	6543.74232	994.786251	375.9939	7463.767	5623.717808	1631	6.787	DEGRADATION
16.699	4967.234	373.699844	167.123651	5431.244	4503.223	9679.04632	1512.24202	571.5738	11077.64	8280.454702	3143	7.789	DEGRADATION
22.999	6458.976	419.871205	187.7721111	6980.316	5937.636	13146.7378	2036.63968	769.7774	15030.32	11263.15884	4712	7.912	DEGRADATION
29.298	7925.596	483.815154	216.3687146	8526.333	7324.859	17075.8481	2631.08831	994.4579	19509.2	14642.49553	6688	8.440	DEGRADATION
35.598	9317.713	554.125626	247.8125136	10005.75	8629.673	20868.5714	3365.93508	1272.204	23981.54	17755.59841	9150	8.991	DEGRADATION
41.897	10530.01	733.99861	328.2541574	11441.39	9618.629	24466.737	4239.77198	1602.483	28387.87	20545.59907	11551	8.912	DEGRADATION
48.196	11787.31	838.518678	374.9969527	12828.47	10746.15	28065.5104	4845.44719	1831.407	32546.8	23584.21584	13937	8.520	DEGRADATION
54.496	13055.81	834.414213	373.1613805	14091.87	12019.75	31672.774	5156.70734	1949.052	36441.94	26903.61163	16278	8.708	DEGRADATION
60.795	14283.39	828.880177	370.6864842	15312.58	13254.2	35161.9778	5427.12996	2051.262	40181.24	30142.7161	18617	9.381	DEGRADATION
67.095	15460.64	810.94065	362.6636836	16467.56	14453.72	38500.3717	5668.22216	2142.387	43742.61	33258.13671	20879	10.016	DEGRADATION
73.394	16662.98	769.230589	344.0103776	17618.11	15707.85	41713.3664	5887.57885	2225.296	47158.47	36268.2602	23040	10.603	DEGRADATION
79.694	17806.66	754.560907	337.4498961	18743.57	16869.74	44827.6386	6109.40305	2309.137	50477.9	39177.37906	25050	11.125	DEGRADATION
85.993	18925.38	746.447483	333.8214627	19852.21	17998.54	47818.8242	6330.04739	2392.533	53673.15	41964.5025	27021	11.579	DEGRADATION
92.293	19952	786.583553	351.7708588	20928.68	18975.33	50622.3606	6755.02237	2553.158	56869.72	44375.0023	28893	11.961	DEGRADATION
98.592	21032.29	758.105063	339.0348912	21973.6	20090.97	53439.023	6923.81423	2616.956	59842.49	47035.55812	30670	11.900	DEGRADATION
104.891	22078.73	715.415504	319.9435398	22967.04	21190.42	56165.7949	7073.00201	2673.343	62707.24	49624.35433	32407	12.281	DEGRADATION
111.191	23132.01	696.423062	311.4498614	23996.73	22267.28	58841.9527	7221.08879	2729.315	65520.35	52163.55451	34087	12.660	DEGRADATION
117.49	24129.29	651.001375	291.1366655	24937.62	23320.97	61419.2355	7342.45807	2775.188	68209.88	54628.58943	35710	12.999	DEGRADATION
123.79	25146.24	650.459233	290.8942122	25953.89	24338.59	63967.4551	7476.29996	2825.776	70881.88	57053.02582	37290	13.364	DEGRADATION
130.089	26142.37	662.331614	296.2037025	26964.77	25319.98	66439.434	7600.37364	2872.671	73468.61	59410.2556	38821	13.666	DEGRADATION
136.389	27124.55	688.475714	307.8956993	27979.41	26269.7	68847.4332	7717.02812	2916.762	75984.5	61710.36728	40297	13.954	DEGRADATION
142.688	28105.11	731.16572	326.9872507	29012.97	27197.25	71209.5475	7830.03714	2959.476	78451.13	63967.96567	41723	14.225	DEGRADATION
148.988	29075.8	777.264842	347.6034045	30040.91	28110.7	73522.8568	7944.06871	3002.576	80869.9	66175.81324	43104	14.477	DEGRADATION
155.287	30038.56	847.681246	379.0945778	31091.1	28986.02	75763.5892	8044.89373	3040.684	83203.88	68323.29798	44447	14.705	DEGRADATION
161.586	31001.76	922.943692	412.752967	32147.75	29855.77	77962.4971	8138.26216	3075.974	85489.14	70435.85445	45725	14.922	DEGRADATION
167.886	31972.59	999.845457	447.1444816	33214.06	30731.12	80142.1407	8233.50662	3111.973	87756.87	72527.41158	46961	15.131	DEGRADATION
174.185	32935.29	1081.32597	483.5836766	34277.94	31592.65	82265.3699	8319.93465	3144.64	89960.03	74570.70812	48170	15.321	DEGRADATION
180.485	33887.76	1163.43212	520.3026626	35332.35	32443.16	84351.8789	8406.11419	3177.213	92126.24	76577.51427	49330	15.505	DEGRADATION
186.784	34828.35	1243.2293	555.9890454	36372.02	33284.67	86380.9698	8483.54742	3206.48	94226.95	78534.99128	50464	15.674	DEGRADATION
193.084	35758.64	1319.63946	590.1607092	37397.19	34120.09	88355.9658	8548.88897	3231.176	96262.38	80449.55639	51553	15.841	DEGRADATION
199.383	36668.62	1397.47601	624.9702721	38403.82	34933.42	90299.4367	8607.5529	3253.349	98260.1	82338.77218	52597	16.013	DEGRADATION
205.683	37580.2	1470.80922	657.7658813	39406.46	35753.95	92220.9111	8675.25194	3278.937	100244.2	84197.63536	53631	16.189	DEGRADATION
211.982	38470.09	1552.65724	694.3694273	40397.97	36542.2	94086.271	8725.9788	3298.11	102156.5	86016.0807	54641	16.339	DEGRADATION
218.281	39371.22	1634.68827	731.0548178	41400.96	37341.48	95944.3934	8780.08589	3318.561	104064.6	87824.16235	55616	16.501	DEGRADATION
224.581	40291.05	1700.69708	760.5748565	42402.75	38179.35	97767.5601	8835.9251	3339.666	105939.4	89595.68638	56573	16.648	DEGRADATION
230.88	41252.63	1715.80576	767.3316625	43383.09	39122.17	99644.9927	8905.84936	3366.095	107881.5	91408.44974	57477	16.781	DEGRADATION
237.18	42237.26	1697.64181	759.2084973	44345.17	40129.36	101544.523	8986.09838	3396.426	109855.3	93233.76173	58392	16.913	DEGRADATION
243.479	43225.7	1669.26387	746.5174963	45298.37	41153.03	103462.435	9072.0658	3428.919	111852.7	95072.16713	59307	17.041	DEGRADATION
249.779	44292.22	1622.7959	725.7363874	46307.19	42277.25	105452.527	9187.19857	3472.435	113949.3	96955.77879	60237	17.165	DEGRADATION
256.078	45346.81	1600.09505	715.5842617	47333.59	43360.03	107406.323	9303.75554	3516.489	116010.9	98801.77817	61160	17.241	DEGRADATION
264.379	46360.38	1795.76149	803.0889548	48590.11	44130.64	109424.101	9430.34463	3564.335	118145.7	100702.48	62060	17.294	DEGRADATION
											63064	17.260	DEGRADATION

TABLE C-1 CONT DATA FOR DETERMINING JP-8 DEGRADATION

Time (Hrs)	MEAN Blank Soil O2	STD DEV	STD ERROR	UPPER 95% C.I. Blank Soil O2	LOWER 95% C.I. Blank Soil O2	MEAN JP-8 & Soil O2	STD DEV	STD ERROR	UPPER 95% C.I. JP-8 & Soil O2	LOWER 95% C.I. JP-8 & Soil O2	$\overline{X}_{JP8} - \overline{X}_{SOIL}$	Calc T Val (|T Crit| = 2.228)	DEGRADATION/ INHIBITION/ NO EFFECT
270.679	47297.99	1871.73015	837.0631698	49622.06	44973.93	111217.282	9514.03418	3595.967	120016.3	102418.2613	63919	17.312	DEGRADATION
276.978	48198.12	1921.16789	859.1723999	50583.57	45812.67	112922.954	9620.47359	3636.197	121820.4	104025.4929	64725	17.323	DEGRADATION
283.278	49116.71	1960.76507	876.880798	51551.32	46682.09	114608.867	9728.70894	3677.106	123606.4	105611.3054	65492	17.325	DEGRADATION
289.577	50038.58	2055.35452	919.182485	52590.64	47486.51	116279.935	9794.39498	3701.933	125338.2	107221.6238	66241	17.366	DEGRADATION
295.877	50947.18	2160.68754	966.288843	53630.04	48264.33	117922.036	9863.16736	3727.927	127044	108800.1213	66975	17.391	DEGRADATION
302.176	51847.53	2273.15242	1016.584665	54670.03	49025.04	119551.467	9932.22198	3754.027	128737.2	110365.6867	67704	17.408	DEGRADATION
308.476	52746.69	2400.80235	1073.671451	55727.69	49765.7	121156.81	9999.48297	3779.449	130404.8	111908.8239	68410	17.412	DEGRADATION
314.775	53566.8	2471.87944	1105.458093	56636.05	50497.55	122551.951	10023.5634	3788.551	131822.2	113281.6937	68985	17.480	DEGRADATION
321.074	54377.72	2555.43092	1142.82345	57550.71	51204.73	123944.976	10053.836	3799.993	133243.2	114646.7217	69567	17.532	DEGRADATION
327.374	55254.83	2657.17015	1188.322618	58554.15	51955.51	125130.685	10042.0577	3795.541	134418	115843.3241	69876	17.569	DEGRADATION
333.673	56058.75	2745.51883	1227.833347	59467.77	52649.74	126470.587	10072.8209	3807.168	135786.4	117154.7747	70412	17.602	DEGRADATION
339.973	56894.69	2835.59133	1268.114995	60415.55	53373.83	127636.481	10088.0969	3812.942	136966.4	118306.541	70742	17.605	DEGRADATION
346.272	57695.29	2937.02828	1313.478976	61342.1	54048.48	128963.544	10127.2016	3827.722	138329.7	119597.4381	71268	17.611	DEGRADATION
352.572	58448.95	3020.04054	1350.60319	62198.83	54699.06	130084.159	10162.4536	3841.046	139482.9	120685.4497	71635	17.594	DEGRADATION
358.871	59242.01	3107.06976	1389.52384	63099.95	55384.06	131356.857	10220.447	3862.966	140809.2	121904.513	72115	17.566	DEGRADATION
365.171	59973.99	3197.99184	1430.185428	63944.83	56003.15	132461.982	10277.5034	3884.531	141967.1	122956.8696	72488	17.512	DEGRADATION
371.47	60755.94	3292.81382	1472.591107	64844.52	56667.36	133708.375	10338.7063	3907.664	143270.1	124146.6591	72952	17.470	DEGRADATION
377.769	61502.43	3304.18641	1477.677087	65605.12	57399.73	134742.515	10454.5783	3951.459	144411.4	125073.6358	73240	17.361	DEGRADATION
384.069	62277.11	3381.81824	1512.395093	66476.2	58078.02	135953.161	10530.9609	3980.329	145692.7	126213.6397	73676	17.303	DEGRADATION
390.368	63046.59	3499.6456	1565.089093	67391.99	58701.2	137165.682	10588.6675	4002.14	146958.6	127372.7911	74119	17.248	DEGRADATION
396.668	63821.08	3625.04422	1621.16906	68322.17	59319.98	138362.035	10644.6885	4023.314	148206.7	128517.3333	74541	17.185	DEGRADATION
402.968	66018.59	4429.54299	2557.39784	77022.2	55014.99	136073.821	8660.9047	4330.452	149855.3	122292.3759	70055	13.930	DEGRADATION
409.268	66799.82	4582.87201	2645.922388	78184.31	55415.32	137189.375	8624.04886	4312.024	150912.2	123466.5763	70390	13.913	DEGRADATION
415.568	67573.3	4724.72874	2727.823411	79310.19	55836.42	138318.074	8601.91693	4300.958	152005.7	124630.4922	70745	13.890	DEGRADATION

Appendix D STATISTICAL DATA FOR RATIO OF O_2/ CO_2

The tables and figures in this appendix summarize the mean oxygen consumption and carbon dioxide evolution from each treatment, along with the respective ratio of O_2/CO_2.

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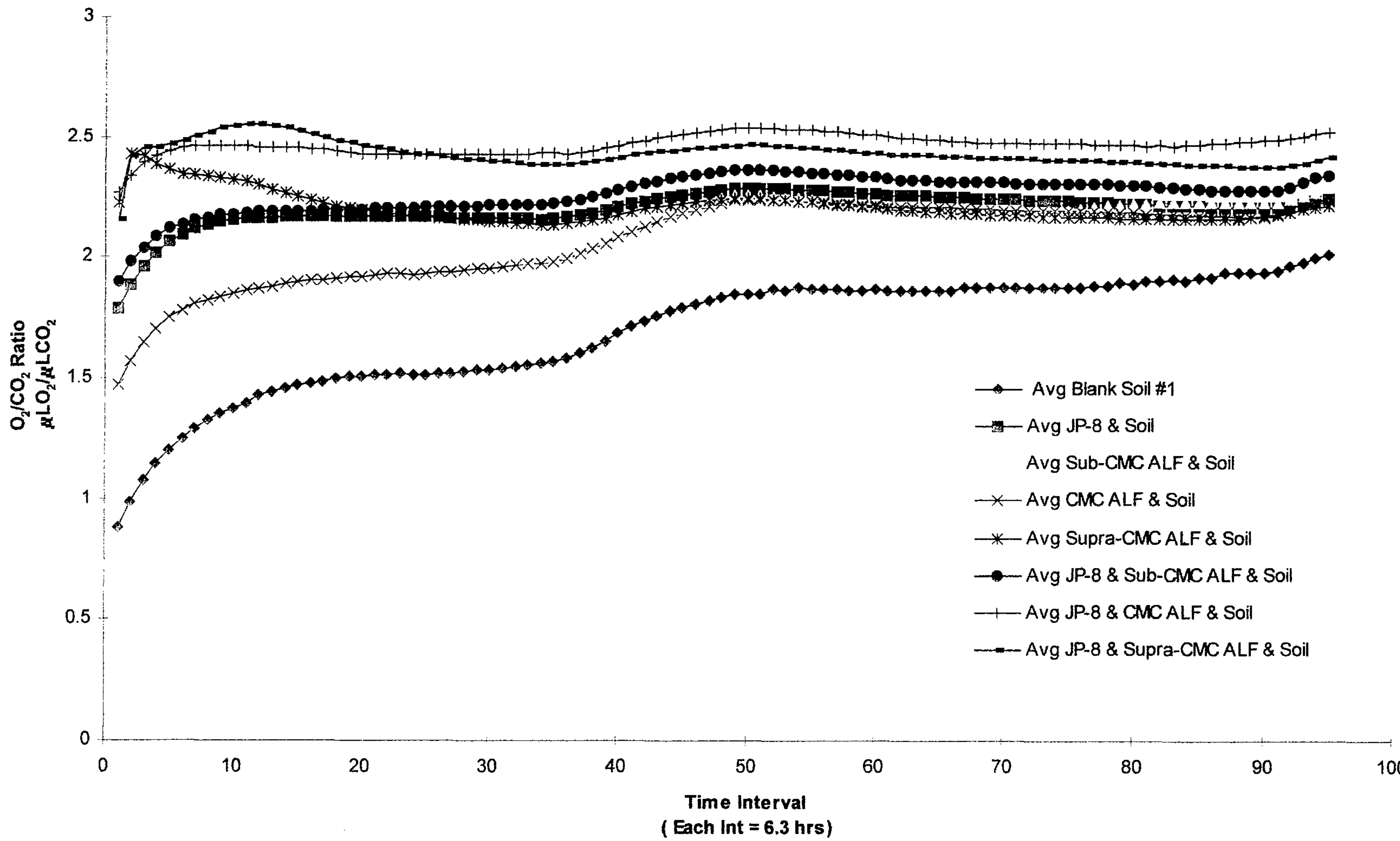
Table D-1 Mean Oxygen Uptake, Carbon Dioxide Evolved, and O_2/CO_2 Ratio Comparisons

Interval	Avg Blank Soil #1			Avg JP-8 & Soil			Avg Sub-CMC ALF & Soil			Avg CMC ALF & Soil			Avg Supra-CMC ALF & Soil			Avg JP-8 & Sub-CMC ALF & Soil			Avg JP-8 & CMC ALF & Soil			Avg JP-8 & Supra-CMC ALF & Soil		
	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2
1	1527	1737	0.8791	3608	2019	1.7866	2455	1582	1.552	2702.5	1833	1.4744	4655	2092	2.2257	3751.5	1975	1.8995	4623	2041	2.2656	4531	2100	2.1573
2	3029	3085	0.9818	7262	3851	1.8856	4673	2866	1.6305	5238.5	3340	1.5684	10532	4339	2.4276	7550	3816	1.9785	9334.5	3993	2.3377	10472	4328	2.4199
3	4483	4182	1.0720	10918	5576	1.9579	6763	3932	1.7202	7579	4602	1.6471	15851	6547	2.4213	11280	5530	2.0399	13975	5844	2.3913	15946	6498	2.4538
4	5873	5145	1.1415	14650	7274	2.0139	8800	4892	1.799	9752	5721	1.7046	20696	8669	2.3874	15019	7192	2.0883	18554	7668	2.4197	21171	8614	2.4579
5	7234	6026	1.2005	18395	8916	2.0632	10691	5772	1.8524	11792	6747	1.7477	25281	10701	2.3625	18741	8840	2.12	23057	9437	2.4434	26347	10667	2.4699
6	8550	6855	1.2473	22112	10562	2.0934	12310	6601	1.8649	13695	7704	1.7777	29719	12663	2.3469	22396	10476	2.1378	27430	11183	2.4528	31555	12715	2.4818
7	9828	7627	1.2886	25821	12182	2.1196	13774	7370	1.8691	15508	8599	1.8035	34075	14548	2.3423	26019	12074	2.155	31729	12892	2.4612	36915	14757	2.5015
8	11066	8363	1.3232	29458	13794	2.1355	15375	8116	1.8944	17229	9450	1.8232	38292	16376	2.3384	29560	13649	2.1658	35899	14572	2.4636	42362	16805	2.5208
9	12271	9078	1.3517	33019	15369	2.1484	16852	8840	1.9064	18873	10268	1.838	42315	18148	2.3317	33028	15188	2.1746	39991	16233	2.4636	47868	18864	2.5375
10	13431	9762	1.3758	36468	16916	2.1559	18330	9542	1.9209	20441	11047	1.8504	46055	19820	2.3237	36375	16687	2.1799	43944	17848	2.4621	53290	20914	2.5481
11	14558	10417	1.3975	39811	18417	2.1617	19925	10219	1.9498	21951	11789	1.8621	49490	21390	2.3137	39630	18144	2.1842	47804	19430	2.4604	58562	22930	2.5539
12	15800	11061	1.4284	43049	19901	2.1632	21674	10878	1.9924	23420	12512	1.8719	52631	22882	2.3001	42925	19604	2.1896	51582	20981	2.4585	63630	24913	2.554
13	16891	11702	1.4434	46255	21359	2.1656	23150	11544	2.0055	24851	13223	1.8794	55482	24302	2.283	46034	21012	2.1908	55276	22506	2.4561	68489	26891	2.5469
14	17951	12314	1.4578	49352	22770	2.1674	24333	12163	2.0006	26233	13894	1.8881	58080	25599	2.2689	49036	22371	2.1919	58860	23978	2.4548	73079	28797	2.5377
15	19003	12934	1.4692	52403	24154	2.1695	25512	12801	1.993	27602	14570	1.8944	60489	26854	2.2526	51992	23707	2.1931	62402	25445	2.4524	77461	30683	2.5246
16	20036	13539	1.4799	55361	25506	2.1705	26694	13403	1.9916	28938	15220	1.9013	62736	28017	2.2392	54865	25015	2.1933	65850	26869	2.4508	81632	32491	2.5124
17	21052	14139	1.4889	58258	26849	2.1698	27867	14010	1.9892	30239	15862	1.9064	64853	29132	2.2262	57675	26261	2.1962	69220	28285	2.4473	85593	34254	2.4988
18	22056	14733	1.4970	61075	28156	2.1692	29025	14607	1.9871	31520	16493	1.9111	66876	30193	2.2149	60414	27485	2.1981	72525	29698	2.4421	89393	35955	2.4863
19	23044	15319	1.5043	63813	29432	2.1681	30148	15211	1.982	32776	17109	1.9157	68804	31202	2.2052	63069	28717	2.1962	75733	31111	2.4343	93026	37602	2.4739
20	24014	15901	1.5102	66476	30652	2.1687	31254	15781	1.9805	34017	17703	1.9215	70663	32163	2.197	65660	29853	2.1994	78856	32455	2.4297	96511	39172	2.4637
21	24964	16489	1.5140	69039	31841	2.1683	32338	16328	1.9805	35231	18293	1.9259	72455	33087	2.1898	68151	30958	2.2014	81876	33739	2.4268	99851	40684	2.4543
22	25897	17073	1.5168	71529	32988	2.1684	33420	16857	1.9826	36433	18885	1.9292	74198	33972	2.1841	70582	32026	2.2039	84823	34960	2.4263	103075	42141	2.4459
23	26820	17671	1.5177	73949	34094	2.1689	34506	17383	1.9851	37624	19503	1.9292	75885	34846	2.1778	72942	33057	2.2065	87683	36141	2.4261	106184	43557	2.4378
24	27740	18294	1.5163	76287	35196	2.1675	35547	17901	1.9857	38786	20113	1.9285	77532	35713	2.171	75228	34078	2.2075	90449	37299	2.425	109196	44947	2.4294
25	28617	18859	1.5174	78490	36197	2.1684	36543	18369	1.9894	39924	20647	1.9337	79119	36481	2.1688	77393	35017	2.2101	93094	38362	2.4267	112091	46221	2.4251
26	29486	19414	1.5188	80624	37201	2.1673	37532	18855	1.9906	41030	21177	1.9375	80659	37269	2.1643	79497	35945	2.2116	95661	39418	2.4269	114869	47481	2.4192
27	30363	19947	1.5222	82713	38203	2.1651	38518	19334	1.9922	42146	21699	1.9423	82197	38053	2.1601	81562	36866	2.2124	98182	40463	2.4265	117610	48726	2.4137
28	31225	20454	1.5266	84711	39142	2.1642	39470	19806	1.9929	43237	22205	1.9472	83685	38807	2.1564	83542	37739	2.2137	100599	41453	2.4268	120258	49917	2.4092
29	32085	20946	1.5318	86689	40072	2.1633	40419	20272	1.9938	44324	22707	1.952	85159	39558	2.1528	85506	38602	2.2151	102997	42438	2.427	122858	51091	2.4047
30	32943	21429	1.5373	88604	40966	2.1628	41363	20741	1.9943	45405	23208	1.9564	86608	40297	2.1493	87405	39431	2.2167	105322	43384	2.4277	125394	52237	2.4005
31	33810	21902	1.5437	90461	41852	2.1614	42297	21194	1.9957	46480	23692	1.9618	88046	41019	2.1465	89252	40250	2.2175	107593	44315	2.4279	127884	53360	2.3966
32	34679	22378	1.5497	92321	42732	2.1604	43233	21653	1.9967	47548	24182	1.9663	89469	41747	2.1431	91097	41064	2.2184	109855	45238	2.4284	130349	54480	2.3926
33	35540	22844	1.5558	94132	43584	2.1598	44176	22108	1.9982	48617	24664	1.9712	90875	42465	2.14	92896	41857	2.2194	112063	46130	2.4293	132770	55576	2.389
34	36392	23288	1.5627	95893	44411	2.1592	45090	22544	2.0001	49665	25125	1.9767	92252	43153	2.1378	94652	42621	2.2208	114240	46982	2.4316	135123	56632	2.386
35	37252	23717	1.5707	97644	45184	2.161	46024	22959	2.0047	50732	25565	1.9845	93628	43816	2.1368	96382	43338	2.224	116374	47784	2.4354	137433	57641	2.3843
36	38161	24082	1.5846	99307	45797	2.1684	46983	23319	2.0148	51814	25946	1.997	95009	44386	2.1405	98033	43936	2.2313	117771	48496	2.4285	139677	58549	2.3857
37	39234	24460	1.6040	101101	46458	2.1762	48102	23698	2.0298	53050	26349	2.0134	96553	44983	2.1464	99839	44563	2.2404	119929	49253	2.4349	142079	59428	2.3908
38	40432	24832	1.6282	102979	47112	2.1858	49361	24073	2.0505	54412	26748	2.0343	98220	45573	2.1552	101732	45184	2.2515	122168	50003	2.4432	144577	60297	2.3977
39	41736	25205	1.6559	104947	47767	2.1971	50731	24451	2.0748	55871	27151	2.0578	99986	46166	2.1658	103719	45807	2.2643	124473	50748	2.4528	147141	61160	2.4059
40	43101	25569	1.6857	107002	48407	2.2105	52180	24819	2.1024	57387	27544	2.0835	101812	46741	2.1782	105787	46416	2.2791	126839	51476	2.464	149758	62005	2.4153
41	44435	25925	1.7140	109018	49024	2.2238	53593	25171	2.1291	58864	27918	2.1084	103600	47296	2.1905	107797	47003	2.2934	129147	52171	2.4755	152311	62823	2.4245
42	45743	26295	1.7396	111024	49658	2.2358	54988	25533	2.1536	60309	28296	2.1314	105352	47860	2.2013	109785	47596	2.3066	131425	52872	2.4857	154831	63656	2.4323
43	46974	26676	1.7609	112940	50274	2.2465	56310	25901	2.1741	61683	28672	2.1513	107035	48431	2.2101	111678	48178	2.318	133611	53547	2.4952	157262	64480	2.4389
44	48134	27054	1.7792	114741	50883	2.255	57557	26266	2.1913	62973	29038	2.1686	108637	48984	2.2178	113467	48756	2.3272	135693	54202	2.5035	159583	65285	2.4444
45	49262	27434	1.7957	116480	51471	2.263	58771	26628	2.2071	64228	29401	2.1846	110199	49535	2.2247	115188	49315	2.3358	137702	54834	2.5113	161840	66072	2.4495
46	50368	27822	1.8104	118178	52060	2.27	59962	26995	2.2212	65455	29767	2.1989	111733	50090	2.2306	116859	49874	2.3431	139673	55467	2.5182	164063	66871	2.4534
47	51468	28218	1.8239	119862	52647	2.2767	61132	27366	2.2339	66667	30137	2.2122	113253	50646	2.2362	118514	50435	2.3498	141628	56099	2.5246	166244	67661	2.457
48	52569	28602	1.8379	121554	53223	2.2839	62313	27726	2.2475	67880	30493	2.2261	114768	51180	2.2424	120166	50979	2.3572	143569	56718	2.5313	168406	68426	2.4611
49	53687	29009	1.8507	123226	53787	2.291	63509	28100	2.2601	69105	30863	2.2391	116295	51743	2.2476	121804	51517	2.3644	145505	57334	2.5378	170560	69194	2.4649

Table D-1 Mean Oxygen Uptake, Carbon Dioxide Evolved, and O_2/CO_2 Ratio Comparisons

Interval	Avg Blank Soil #1			Avg JP-8 & Soil			Avg Sub-CMC ALF & Soil			Avg CMC ALF & Soil			Avg Supra-CMC ALF & Soil			Avg JP-8 & Sub-CMC ALF & Soil			Avg JP-8 & CMC ALF & Soil			Avg JP-8 & Supra-CMC ALF & Soil		
	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2
50	54371	29375	1.8509	124423	54291	2.2918	64249	28432	2.2597	69903	31190	2.2412	117356	52223	2.2472	122985	51998	2.3652	146981	57877	2.5395	171331	69375	2.4696
51	55064	29763	1.8501	125647	54835	2.2914	65005	28792	2.2577	70717	31541	2.2421	118433	52740	2.2456	124189	52509	2.3651	148499	58467	2.5399	172989	70094	2.468
52	56053	30024	1.8669	126403	55222	2.289	65465	29049	2.2536	71211	31795	2.2397	119080	53131	2.2413	124923	52881	2.3624	149428	58900	2.537	173994	70605	2.4643
53	56733	30413	1.8654	127570	55777	2.2872	66176	29426	2.2489	71998	32162	2.2386	120108	53675	2.2377	126050	53412	2.3599	150877	59510	2.5353	175567	71333	2.4612
54	57545	30691	1.8750	128336	56188	2.284	66654	29701	2.2442	72508	32434	2.2356	120781	54083	2.2333	126803	53799	2.357	151824	59975	2.5314	176585	71872	2.4569
55	58219	31093	1.8724	129511	56764	2.2816	67388	30093	2.2393	73303	32816	2.2338	121841	54626	2.2305	127947	54339	2.3546	153294	60612	2.5291	178162	72612	2.4536
56	58634	31358	1.8698	130221	57163	2.2781	67827	30367	2.2336	73767	33082	2.2298	122454	55029	2.2253	128627	54721	2.3506	154168	61064	2.5247	179096	73127	2.4491
57	59294	31746	1.8678	131316	57715	2.2752	68545	30761	2.2283	74527	33453	2.2278	123452	55567	2.2217	129701	55255	2.3473	155580	61684	2.5222	180599	73833	2.446
58	59709	32027	1.8643	132059	58117	2.2723	69010	31054	2.2223	75015	33731	2.2239	124105	55978	2.217	130414	55646	2.3436	156472	62152	2.5176	181549	74355	2.4416
59	60370	32427	1.8617	133144	58676	2.2691	69724	31451	2.2169	75778	34111	2.2215	125134	56513	2.2142	131479	56205	2.3393	157862	62804	2.5136	183031	75064	2.4383
60	61033	32694	1.8668	133830	59067	2.2657	70155	31732	2.2109	76234	34379	2.2175	125741	56918	2.2092	132135	56607	2.3343	158707	63274	2.5082	183924	75559	2.4342
61	61668	33070	1.8648	134870	59608	2.2626	70830	32121	2.2051	76956	34745	2.2149	126709	57462	2.2051	133125	57166	2.3288	160025	63922	2.5034	185313	76235	2.4308
62	62311	33442	1.8633	135925	60118	2.261	71524	32495	2.2011	77691	35105	2.2131	127692	58024	2.2007	134133	57672	2.3258	161339	64526	2.5004	186695	76877	2.4285
63	62949	33803	1.8622	136939	60626	2.2587	72204	32859	2.1974	78401	35453	2.2114	128660	58586	2.1961	135103	58160	2.3229	162629	65125	2.4972	188056	77505	2.4264
64	63573	34159	1.8611	137976	61132	2.257	72877	33215	2.1941	79108	35801	2.2097	129620	59132	2.1921	136081	58640	2.3206	163918	65749	2.4931	189413	78127	2.4244
65	64195	34507	1.8603	139000	61627	2.2555	73562	33561	2.1919	79821	36141	2.2086	130579	59658	2.1888	137054	59102	2.3189	165208	66371	2.4892	190763	78731	2.423
66	64827	34847	1.8603	140049	62114	2.2547	74266	33902	2.1906	80552	36479	2.2082	131553	60169	2.1864	138039	59551	2.318	166507	66962	2.4866	192123	79325	2.422
67	65848	35103	1.8759	140726	62488	2.2521	74730	34164	2.1874	81026	36742	2.2053	132202	60566	2.1828	138704	59893	2.3159	167352	67417	2.4823	193002	79784	2.4191
68	66531	35479	1.8752	141817	63034	2.2499	75478	34540	2.1853	81806	37127	2.2034	133264	61123	2.1803	139766	60383	2.3147	168739	68053	2.4795	194435	80447	2.4169
69	67204	35846	1.8748	142882	63572	2.2476	76225	34910	2.1835	82569	37498	2.2019	134311	61658	2.1783	140802	60856	2.3137	170079	68650	2.4775	195829	81080	2.4152
70	67871	36209	1.8744	143973	64091	2.2464	76977	35272	2.1824	83338	37861	2.2011	135352	62183	2.1767	141850	61307	2.3138	171435	69215	2.4768	197219	81693	2.4142
71	68596	36566	1.8760	144980	64614	2.2438	77878	35628	2.1859	84065	38212	2.2	136369	62687	2.1754	142824	61756	2.3127	172719	69762	2.4758	198556	82290	2.4129
72	69244	36927	1.8752	146030	65142	2.2417	78692	35994	2.1863	84805	38565	2.199	137387	63199	2.1739	143833	62205	2.3122	174023	70310	2.4751	199890	82891	2.4115
73	69907	37276	1.8754	147042	65650	2.2398	79518	36339	2.1882	85537	38905	2.1986	138396	63692	2.1729	144811	62642	2.3117	175297	70838	2.4746	201201	83473	2.4104
74	70551	37617	1.8755	148057	66146	2.2383	80433	36662	2.1939	86268	39237	2.1986	139392	64175	2.1721	145787	63065	2.3117	176569	71352	2.4746	202506	84041	2.4096
75	71188	37975	1.8746	149071	66648	2.2367	81319	36992	2.1983	86990	39578	2.198	140387	64672	2.1708	146760	63495	2.3114	177826	71868	2.4743	203791	84618	2.4084
76	71833	38330	1.8741	150078	67149	2.235	82351	37314	2.207	87714	39921	2.1972	141422	65159	2.1704	147718	63929	2.3107	179074	72384	2.4739	205068	85195	2.407
77	72758	38569	1.8864	150709	67495	2.2329	82954	37539	2.2098	88154	40162	2.195	142068	65520	2.1683	148309	64241	2.3086	179836	72750	2.472	205851	85599	2.4048
78	73392	38926	1.8854	151695	67995	2.231	83939	37861	2.217	88860	40516	2.1932	143088	66023	2.1672	149225	64689	2.3068	181046	73271	2.4709	207090	86182	2.4029
79	74282	39185	1.8957	152352	68362	2.2286	84590	38102	2.2201	89327	40781	2.1904	143759	66398	2.1651	149849	65019	2.3047	181839	73665	2.4685	207903	86618	2.4002
80	74944	39568	1.8941	153366	68889	2.2263	85600	38444	2.2266	90073	41165	2.1881	144824	66913	2.1644	150815	65484	2.3031	183109	74211	2.4674	209177	87234	2.3979
81	75778	39817	1.9032	154004	69247	2.224	86205	38681	2.2286	90521	41425	2.1852	145457	67284	2.1618	151396	65825	2.3	183877	74594	2.465	209958	87657	2.3952
82	76407	40173	1.9019	154947	69736	2.2219	87133	39026	2.2327	91214	41783	2.1831	146469	67777	2.1611	152295	66301	2.297	185190	75070	2.4669	211154	88231	2.3932
83	77170	40420	1.9092	155577	70087	2.2198	87747	39272	2.2344	91659	42034	2.1806	147134	68127	2.1597	152889	66644	2.2941	185978	75419	2.4659	211920	88645	2.3907
84	77794	40778	1.9077	156539	70572	2.2182	88632	39652	2.2352	92363	42379	2.1795	148249	68596	2.1612	153813	67129	2.2913	187485	75838	2.4722	213116	89213	2.3889
85	78798	41010	1.9214	157112	70897	2.2161	89117	39907	2.2331	92771	42612	2.1771	148871	68928	2.1598	154336	67468	2.2875	188248	76136	2.4725	213817	89595	2.3865
86	79388	41347	1.9200	157993	71351	2.2143	89858	40233	2.2334	93417	42934	2.1758	149949	69367	2.1617	155158	67923	2.2843	189595	76555	2.4766	214919	90125	2.3847
87	80664	41581	1.9399	158583	71672	2.2126	90320	40479	2.2313	93838	43164	2.174	150607	69684	2.1613	155721	68237	2.2821	190383	76859	2.4771	215629	90502	2.3826
88	81319	41947	1.9386	159506	72158	2.2105	91036	40827	2.2298	94501	43507	2.1721	151787	70126	2.1645	156618	68696	2.2799	191882	77296	2.4824	216755	91066	2.3802
89	81980	42296	1.9382	160412	72608	2.2093	91816	41184	2.2294	95165	43825	2.1715	153240	70533	2.1726	157498	69119	2.2786	193340	77735	2.4872	217874	91588	2.3788
90	82667	42627	1.9393	161304	73043	2.2084	92542	41505	2.2297	95836	44121	2.1721	154620	70940	2.1796	158401	69506	2.279	194742	78170	2.4913	218977	92082	2.3781
91	83545	42937	1.9458	162228	73449	2.2087	93363	41833	2.2318	96680	44398	2.1776	156001	71402	2.1848	159451	69879	2.2818	196139	78655	2.4937	220174	92553	2.3789
92	85210	43293	1.9682	163659	73900	2.2146	94630	42173	2.2439	97910	44719	2.1894	157590	71852	2.1933	161115	70282	2.2924	197854	79164	2.4993	221965	93070	2.3849
93	86559	43613	1.9847	165399	74311	2.2258	96099	42519	2.2602	99294	45008	2.2061	159274	72317	2.2024	163709	70631	2.3178	199688	79675	2.5063	224149	93545	2.3962
94	87917	43934	2.0011	167137	74716	2.237	97570	42857	2.2767	100702	45298	2.2231	160946	72794	2.211	165882	71072	2.334	201848	80124	2.5192	226250	93991	2.4072
95	89276	44253	2.0174	168828	75102	2.248	99079	43199	2.2935	102183	45585	2.2416	162591	73264	2.2192	167696	71494	2.3456	203780	80596	2.5284	228333	94422	2.4182

Figure D.1 Graph Depicting the Ratio of O2/CO2 Over Time



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Vita

Captain John D. Thomas [redacted]. He graduated from Hillsborough High School in 1985, and attended the University of South Florida. He graduated in 1990 with a Bachelor of Science in Mechanical Engineering. From July 1988 to January 1991, John worked at Anheuser Busch, Busch Gardens as an assistant engineer. He was commissioned a 2nd Lieutenant in the US Air Force on 15 December 1990. From January 1991 until August 1991 he worked as environmental consultant. He entered active duty in the US Air Force on 25 September 1991, working as the base mechanical engineer and chief of maintenance engineering at 17 Civil Engineering Squadron, Goodfellow Air Force Base, TX (September 1991 - May 1995). From May 1993 until October 1993 he was TDY in support of Operation Southern Watch, Dhahran, Kingdom of Saudi Arabia, where he served as the CENTAF forward environmental engineer. He was subsequently selected to study for his Masters of Science in Engineering and Environmental Management at the Air Force Institute of Technology from June 1995 until December 1996. Upon completion of the AFIT program he will be assigned to the 18 Civil Engineering Group, Kadena Air Base, Okinawa, Japan.

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13. ABSTRACT *(Maximum 200 words)*

This research effort used an automated respirometer to evaluate the enhancement of JP-8 fuel biodegradation, from surfactant addition. Nonionic surfactants were added to contaminated JP-8 soil, at three levels of treatment. The three levels of treatment included sub-CMC, CMC, and supra-CMC concentrations for each surfactant. The focus of the study was to determine if JP-8 was actually degraded, and if enhancement of JP-8 biodegradation would be achieved from surfactant addition. The respirometer was found to be repeatable within experimental runs. However, reproducibility between experimental runs was not as easy to conclude. The reason for this was that measurement error was introduced from adding surfactants from separate stock solutions. JP-8 biodegradation was proven at all sampling intervals using a statistical hypothesis test about the difference of the means. Enhancement, inhibition, or no effect of surfactant addition was concluded using a statistical hypothesis based on the difference of the mean oxygen uptake, at each sampling interval, for the combined treatments of JP-8 and surfactant, and the individual treatments of JP-8 and surfactant. One level of Alfonic surfactant addition, CMC, resulted in enhancement being concluded.

14. SUBJECT TERMS
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